Influences of environmental and biological factors on song complexity in songbirds

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Frontispiece

A small group of tui (*Prosthemadera novaeseelandiae*) at a feeding station (photo: Anna Arrol, with permission).

“*The Parish I live in is a very abrupt, uneven country, full of hills and woods, and therefore full of birds*”.

(*Gilbert White*)

Abstract

In songbirds, song is important for mate attraction and territory defence. Females of some species preferentially select males that have more complex songs, an honest signal for male fitness. Examining variation in song complexity provides important insights into the evolution of sexually-selected vocal characteristics. In this thesis, hypotheses examining song complexity variation and a series of biological and environmental factors were tested. A socially monogamous songbird with highly complex songs and high extra-pair paternity (tui, *Prosthemadera novaeseelandiae*) was selected as the main study model. Firstly, the hypothesis that song complexity in songbird broadcast songs would be higher than in interactive songs was tested. In addition, it was predicted that there would be a positive association between song complexity and extra-pair paternity frequency. This was conducted across 78 songbird species, the most comprehensive analysis in this study area to date. Concordant with the predictions, tui broadcast songs were found to have higher complexity than interactive songs. Furthermore, after controlling for phylogenetic relatedness, a significant positive association between extra-pair paternity frequency and within-song complexity was found across multiple species. Secondly, I tested the hypothesis that tui song complexity would be higher at dawn than at solar noon and dusk. It has previously been established that dawn is a critical period for intensified songbird vocal displays, such as increased song rate. However, little research has been conducted on diurnal variations in song complexity, which was predicted to be higher at dawn. As predicted, both tui song complexity and intrusion rates were significantly greater at dawn than at dusk. In addition, two song complexity variables were inversely correlated with intrusion rate. Thirdly, the hypothesis that male tui would respond more aggressively to more complex songs was tested, to assess whether song complexity plays a role in male-male interactions. Male responses to rival male songs of different degrees of complexity were subsequently examined using playback experiments. Male tui songs with higher complexity evoked stronger and more aggressive intrasexual responses than simple song as predicted. Fourthly, I tested the hypothesis that habitat complexity would correlate positively with tui song complexity. The association between habitat structure and tui song complexity was investigated by comparing male song complexity in two types of habitat: forest remnants with high complexity, and open habitats with lower complexity. As predicted, habitat complexity correlated positively with tui song complexity. Overall, the findings in this thesis provide evidence that several biological and environmental factors are associated with the evolution of song complexity; a socially-selected vocal trait. This study suggests that complex songs in vocally complex songbirds may have evolved under extra-pair paternity, territorial and environmental pressures. It therefore has implications for furthering our understanding of song complexity evolution in songbirds.

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1 General introduction

Tui in flight after feeding on flax nectar (*Phormium tenax*) (photo: Andrew Underwood, with permission).

1.1 Songbird vocal behaviour

Vocal communication is a behavioural trait important for the fitness of animals, especially songbirds (Suborder *Passeri*). It is therefore an important study area within the fields of behavioural ecology and evolutionary biology (Catchpole & Slater 2008; Mason et al. 2017). Evidence indicates that many aspects of song are subject to both inter- and intra-sexual selection and play central roles in songbird mate attraction and territory defence (Catchpole & Slater 2008). Both males and female songbirds may sing however this thesis focuses on male song only.

Females may preferentially select specific vocal features in males, as they may be reliable indicators of male physical or behavioural qualities (Drăgănoiu et al. 2002). For example, females of some species have been shown to be attracted to males exhibiting higher song rates (Alatalo et al. 1990; Nowicki & Searcy 2004), which would therefore potentially give such males more mating opportunities with a larger number of partners (Lattin & Ritchison 2009). There will also likely be directional selection for louder males due to female preferences for this trait (Ritschard et al. 2010) as well as songs with broad bandwidths (Drăgănoiu et al. 2002). Song consistency, the ability to accurately repeat each song type, can also be an important male trait in terms of intersexual selection, as it is positively correlated with reproductive success and dominance ranking in tropical mockingbirds (*Mimus gilvus*, Botero et al. 2009). Multiple song traits are associated with male quality because they can also reflect early-life conditions of individuals during song learning, such as brood size and the level of developmental stress experienced (Buchanan et al. 2004; Botero & De Kort 2013).

In addition to intersexual selection, song can be considered to be under intrasexual selection because it plays a key role in male territory defence (Catchpole & Slater 2008). For example, territorial males increase song amplitude during simulated rival male challenges in the nightingale *Luscinia megarhynchos* (Brumm & Todt 2004). Furthermore, male song output is increased during the female fertile period, functioning to prevent rival males undertaking extra-pair (EP) mating with his partner (Møller 1991). Another song characteristic that plays a role in territory defence is the production of rapid trill sound units (syllables) without a significant narrowing of bandwidth (vocal deviation). There is evidence that the production of fast trills and broad bandwidths are perceived by competitors as honest signals of male quality because the ability to maximise both trills and bandwidth is governed largely by motor constraints on song production (Phillips & Derryberry 2017). Exhibiting singing behaviours that serve to assert territory has several evolutionary advantages for territorial males such as reducing physical conflict thereby minimising the chances of injury. Males thus conserve energy and resources which can then be used to enhance future mate attraction and subsequently reproductive success (Catchpole & Slater 2008).

Song complexity is another vocal behaviour characteristic considered to be under both inter- and intrasexual selection in songbirds (Ballentine et al. 2003; Catchpole & Slater 2008). Song complexity has become a key model within studies on sexual selection in recent years (Garamszegi et al. 2012). Evidence suggests that the females of multiple species will preferentially select males that produce songs that are more complex (Catchpole et al. 1986; Eens et al. 1991). There is also evidence that males will also assess rival male songs based on their level of complexity. For instance, songs that have higher complexity may stimulate a greater number of aggressive approaches by rival males in some species (Mountjoy & Lemon 1991). Song complexity traits are important in an evolutionary biology context since the ability to learn complex songs is genetically heritable from father to son (Woodgate et al. 2013; Croston et al. 2015). From an evolutionary perspective, heritability is critical in order for sexual selection to operate (Trösch et al. 2017). Song complexity is also a permanent trait in some species (Kubli & MacDougall-Shackleton 2014) that incurs costs associated with song nuclei development. These song nuclei are areas within the songbird brain specialised for song learning and production (Nowicki et al. 1998, 2002). Song complexity therefore is an important trait in the study of the evolution of vocal behaviour.

1.1.1 Song complexity

Song complexity can be measured in terms of between-song complexity (e.g. the size of male song or syllable repertoire), and ‘within-song complexity’ (e.g. song length, the number of different syllables within a song, and the number of transitions between syllables) (Eens et al. 1991; Mountjoy & Lemon 1991; Ballentine et al. 2004; Illes et al. 2006; Zhang et al. 2017). In some songbird species, song complexity is more strongly selected by females over performance-based traits such as high song output (Cramer 2013), as the production of complex songs may be associated with male cognitive performance, fitness and reproductive success (Catchpole 1986; Reid et al. 2005; Boogert et al. 2011; Soma & Garamszegi 2011). High song complexity therefore can be considered an honest signal of male quality (Buchanan & Catchpole 2000). Females may assess both between-song complexity and within-song complexity. For example, there is evidence that females preferentially select males with large song and syllable repertoires (Searcy 1992; Searcy & Yasukawa 1996; Buchanan & Catchpole 1997; Reid et al. 2005). In addition, males with large song repertoires are less likely to suffer mate cuckoldry than those with smaller repertoires (Hasselquist et al. 1996; Hasselquist 1998). Moreover, research indicates that song repertoire size is inversely related to the level of male inbreeding (Reid et al. 2005), thus song complexity may be attributable to parental genetic compatibility. Males that sing longer song bouts have also been shown to keep mates for longer periods of time and produce larger clutches than males that sing shorter song bouts (Eens et al. 1991; Mennill et al. 2005). Furthermore, females may selectively copulate with males who sing a larger number of different syllables within a song (Catchpole et al. 1986). The females of many songbird species may preferentially select males that produce syntactically complex songs (Woodgate et al. 2011), as they are energetically-expensive signals reflecting genotypic and phenotypic quality (Nowicki et al. 2000; Ballentine 2009). However, previous evidence suggests that complex songs may have evolved under intersexual selection pressures in monogamous species only. In polygynous species (males with more than one social partner), songs may have evolved under intrasexual pressures to become shorter, simpler and more stereotyped, which function more for male-male interactions in a territorial context (Catchpole 1980). Collectively, this evidence demonstrates that songbird song complexity can be an honest signal reflecting multiple measures of male quality.

Previous studies have found that male between-song complexity, in terms of song repertoire size, can be positively correlated with successful territory acquisition, defence and tenure (Catchpole & Slater 2008; Potvin et al. 2015). Furthermore, evidence suggests males of some species (e.g. starling *Sturnus vulgaris*) that produce simpler songs with fewer phrase types (complexes of notes or syllables within a song, Nelson & Marler 1994) are more likely to suffer territorial invasions than those that produce complex songs (Falls 1988; Mountjoy & Lemon 1991). Though the reasons for this are unclear, it has been suggested males assess and perceive high song complexity as a signal that reflects territorial defence capabilities (Hesler et al. 2011). This relates back to mating success because territory holders that have lower song complexity and lower territory defence abilities have a greater chance of suffering partner cuckoldry by rival males which would subsequently also gain extra-pair copulations. However, the effect of within-song complexity characteristics such as syllable diversity on territory defence is still yet to be fully understood.

1.1.2 Song complexity variation

Song complexity varies markedly among species, with some producing monotonous patterns composed merely of a few syllables (Catchpole 1980). For example, the zebra finch (*Taeniopygia guttata*) sings highly stereotyped songs where only one song type with slight variations is produced (Glaze & Troyer 2006). Similarly, the swamp sparrow (*Melospiza georgiana*) produces songs composed of a single syllable repeated at a constant rate (Gould 2004). In contrast, other species have highly complex songs with a large repertoire of syllables organised in non-random fashion with discernible patterns (Sasahara et al. 2012). The European starling (*Sturnus vulgaris*) for example produces many different song types (Moorman & Bolhuis 2013), and the winter wren (*Troglodytes hiemalis*) produces up to 40 different syllables per song (Kroodsma 1980). Kroodsma (1980) claimed the winter wren to be 'the pinnacle of song complexity' due to its high syllable diversity. However, other species (e.g. the superb lyrebird *Menura novaehollandiae*, Dalziell et al. 2013, California thrasher *Toxostoma redivivum*, Sasahara et al. 2012, red-eyed vireo *Vireo olivaceus*, Borror 1981, and common nightingale, Bartsch et al. 2015) have also been documented as having highly complex songs with large syllable and/or song repertoires.

Pronounced interspecific variation in song complexity also has been shown to exist within the same taxonomic family (e.g. honeyeaters, Meliphagidae). The repertoires of noisy miners (*Manorina melanocephala*) and New Holland honeyeaters (*Phylidonyris novaehollandiae*) consist of simple single note whistles (Jurisevic & Sanderson 1994). Red wattlebirds (*Anthochaera carunculata*) and little wattlebirds (*A. chrysoptera*) also sing relatively simple songs, generally consisting of harsh syllables covering several frequencies (Jurisevic & Sanderson 1994). Contrarily, bellbirds (*Anthornis melanura*) and tui (*Prosthemadera novaeseelandiae*) sing complex vocalisations consisting of many different syllables and songs (Brunton & Li 2006; Hill 2011; Hill et al. 2013). This suggests phylogeny alone, cannot explain the variation in the degree of song complexity in different species (i.e. phylogenetic signal). The evolutionary drivers and biological significance of song complexity variation is still uncertain.

Because song complexity can be directly related to male reproductive success in certain species (Hasselquist et al. 1996; Hasselquist 1998; Forstmeier et al. 2002), intraspecific song complexity variation can occur. Both song complexity and reproductive behaviour (e.g. the frequency of extra-pair paternity, EPP) vary hugely across taxa (Catchpole 1980; Taylor et al. 2008; Sasahara et al. 2012). Since song complexity has previously been found to correlate with breeding success it is possible there is an evolutionary association between these two behavioural factors. Sperm competition between males occurs in socially monogamous species that engage in EPP (Birkhead 1998), with the prediction that sperm competition would be greater in those species with high EPP frequency. Furthermore, as song complexity is often strongly selected by females in these species, male song complexity in the males should also be higher, as it reflects an individual’s quality (Catchpole 1986; Reid et al. 2005), physical condition (Spencer et al. 2003; Pfaff et al. 2007; MacDougall-Shackleton et al. 2009), and social status (Bartsch et al. 2015). Some studies have indeed documented significant within-species associations between EPP frequency and certain song complexity characteristics such as song repertoire size and song duration (Hasselquist et al. 1996; Gil et al. 2007). Nevertheless, two major meta-analysis studies concluded that either weak, negligible, or overall nonsignificant relationships exist between EPP frequency and several aspects of song complexity and performance in a wide range of species (Garamszegi & Møller 2004; Soma & Garamszegi 2011). This inconsistency in conclusions suggests examinations of the relationship between EPP frequency and song complexity requires further research.

In addition to reproductive fitness, evidence demonstrates that in multiple songbird species there is an association between song complexity and several other fitness traits. For example, male song complexity is positively correlated with metrics such as the number of grandoffspring produced in addition to male parental care ability i.e. their ability to successfully rear offspring from hatchling to parental care independence (Reid et al. 2005; Bartsch et al. 2015). Furthermore, highly complex songs produced by male swamp sparrows are directly correlated with age and body mass (Ballentine 2009). Females therefore gain immediate, direct, indirect and intergenerational fitness benefits by selecting males that have higher song complexity (Hasselquist et al. 1996; Nowicki et al. 2000; Buchanan et al. 2004; Reid et al. 2005; Bartsch et al. 2015). Song complexity can also reflect the health of a population (Laiolo et al. 2008). For example, in healthy populations of Dupont's larks (*Chersophilus duponti*), individuals had faster growth rates during development, there were more males and higher ratios of young birds to adults that sang a more diverse array of complex songs. Birds from slower growing and less dynamic populations on the other hand were more likely to sing simpler songs (Laiolo et al. 2008). These multiple factors can give rise to variation in intraspecific song complexity.

Intraspecific song complexity variation has also been demonstrated at the macrogeographic level. For example, island populations of some songbird species have been found to have reduced song complexity than their mainland congeners (Baker 1996; Hamao & Ueda 2000; Tu & Severinghaus 2004; Hamao et al. 2013). The founder effect and a lesser scope for immigration and emigration, which can consequently lead to competition reduction within island populations (Baker 1996; Valderrama et al. 2013), are factors that can contribute to a reduction in vocal signal complexity. These findings demonstrate that both demographic and geographical factors are associated with song complexity reduction. Differing degrees of song complexity on a microgeographic spatial scale in areas of differing habitat complexity however is currently unresolved.

Environmental factors may also influence song complexity in songbirds. For example, song duration may be longer in territories within dense forest areas (Van Dongen & Mulder 2006). Furthermore, in American tree sparrows (*Spizella arborea*) song repertoire size was inversely correlated with species-richness within forested habitats (Naugler 1992; Naugler & Ratcliffe 1994). On the other hand, in species-poor areas, American tree sparrows would sing a greater number of different song types (Naugler & Ratcliffe 1994). These factors are consistent with the acoustic competition hypothesis which states that song complexity will increase with decreasing number of species (Naugler & Ratcliffe 1994; Hart et al. 2018). Alternatively, habitat complexity in terms of vegetation biodiversity, was shown to be positively correlated with the acoustic complexity of multiple songbird species in a tropical dry forested habitat, as measured by the acoustic complexity index (Hilje et al. 2017). Acoustic complexity may also influence other songbird vocal behaviours for example, song frequency will generally be lower in closed habitats with greater levels of complexity because higher frequency sounds are more easily scattered by the greater number of dispersing surfaces within forested areas (Morton 1975). In other words, lower frequency sounds have better sound attenuation in forested areas than higher frequency sounds. These aspects highlight that varying environmental conditions may influence songbird vocal behaviour in a variety of different means.

Varying song complexity may also be exhibited within an individual's repertoire (intra-individual variation; Leitão et al. 2006). Chaffinches (*Fringilla coelebs*), for example, may produce songs of differing complexity levels in terms of varying quantities of trill syllables produced per song (Leitão et al. 2006). Furthermore, evidence suggests field sparrows (*Spizella pusilla*), which have just two song types within their repertoire (one complex and one simple song), produce their complex song type more often within specific contexts. For example, their complex song type is produced more during both territorial confrontations, and during dawn, when territorial intrusions are most likely (Nelson & Croner 1991). More vocally complex species, such as tui, have a higher number of syllables within their repertoire and therefore are likely to produce a far wider variety of songs with differing complexity levels than vocally simpler species (Hill et al. 2015). However, whether tui also produce their higher complexity songs during the dawn period is unknown. Since previous studies demonstrate that vocal aspects of some species are intensified at dawn (Erne & Amrhein 2008; Sandoval & Mennill 2014; Quispe et al. 2016), song complexity, a trait known to be important for territory defence in many species, should show the same trend.

In species with large syllable and song repertoires, the function of songs with differing complexity levels is not yet fully established. Evidence implies however that tui song complexity exhibits inter-seasonal variations. Specifically, in the breeding season, songs are more complex in terms of a greater amount of trill syllables than those in the non-breeding season (Hill et al. 2015). This suggests that songs that are more complex function to maximise mate attraction and territory assertion during the breeding season, although this prediction has yet to be empirically tested. Examining the role of song complexity variations within an individual's repertoire helps establish the relationship between structure and function of multiple intra-individual signals (Chaine et al. 2015). It also provides important insights into the evolution of divergent songs within an individual's repertoire, or in other words, the adaptive significance of producing songs with differing complexity levels.

In this thesis, the correlates of songbird song complexity and a series of important biological (EPP and territoriality) and environmental (habitat variations) factors were investigated. Song complexity is a vocal trait key to advancing the understanding of the relationship between vocal behaviour and sexual selection. Examining variation in songbird song complexity also enhances our understanding of sexually-selected complex signals and the factors that at least partly drive their evolution (Boogert et al. 2008). It also advances our understanding of how song is linked to factors such as acoustic adaptation, cultural evolution, and quality signalling (Lynch 1996; Boncoraglio & Saino 2007; Ballentine 2009). Ideal study models to investigate factors associated with song complexity variations are species with high song complexity, since such species have the potential for large variation at the syllable or syntactic level (Kiefer et al. 2006; Briefer et al. 2008; Briefer et al. 2010). Examining song traits that have evolved into extreme forms such as high song complexity helps to gain a greater understanding of the association between function and signal evolution (Funghi et al. 2015).

1.1.3 General methods and summary of song complexity measures

Vocalisations in songbirds include both calls and songs. These can be straightforwardly differentiated, as calls are generally structurally simple and contextual, are often produced in reference to a particular function such as distress signals in addition to being innate (Laiolo et al. 2004). Songs on the other hand are principally more complex and often require the process of learning from conspecifics in a complex interaction between inborn programming and plastic imitation (Gardner et al. 2005; Catchpole & Slater 2008). This thesis focused on songs only, both broadcast songs, which are intended for distant audiences, and interactive songs, used in male-male confrontations and male-female interactions (Titus 1998; Reichard et al. 2011).

Song complexity can be measured using several quantitative variables, for example song and syllable repertoire size, and song versatility (measures of between-song complexity, Doutrelant et al. 2000; Garamszegi & Møller 2004; Zeng et al. 2007).

Syllables are generally considered the basic building blocks of songs (Markowitz et al. 2013). A syllable can be defined as either a continuous tracing on a sound spectrogram; or a tight group of identical sound units (Garamszegi et al. 2003; Boogert et al. 2008; Yu et al. 2017) separated from other syllables by a visible temporal interval ≥ 90 ms (Figure 1.1). The extraction of syllables requires manual inspection of songs and is labour-intensive, however the advent of advanced automated syllable-recognition algorithms is likely to facilitate rapid and conceivably more accurate measures of syllable counts in future efforts (Burkett et al. 2015).

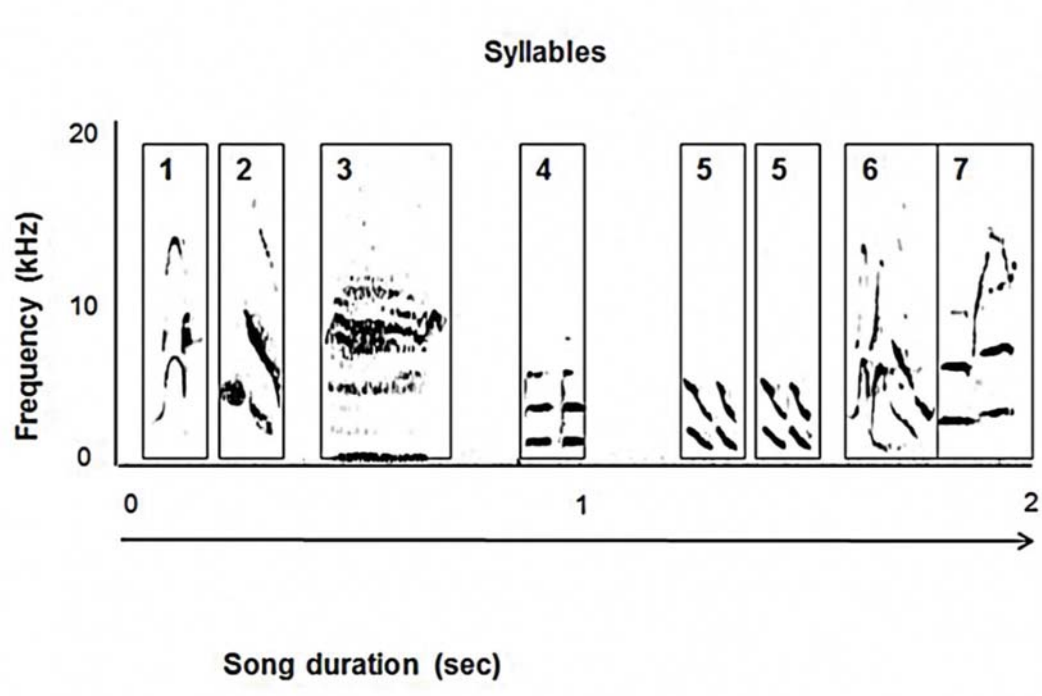


Figure 1.1: A spectrogram of a male tui song highlighting total number of syllables, song duration and syllable diversity. This example has eight syllables in total and seven different syllables (syllable five is repeated). The syllable rate of this song is 8 ÷ 2 sec = 4 syllables per second.

However, a mere count of syllables within a repertoire may not be the most appropriate way to measure song complexity (Gil & Slater 2000). By focusing only on absolute repertoire size, meaningful variation in other variables related to complexity could easily be missed (Leitão et al. 2006), thus using multiple variables that measure within-song complexity could be more important to measure overall complexity (Nowicki & Podos 1993).

Within-song complexity measures include: 1. Song length or duration, 2. Syllable diversity, 3. Syllable rate, 4. Syllable transitions, 5. Total number of syllables per song, 6. Trill duration and proportion. Song length or duration (Boogert et al. 2008) is defined as the length of time from the start of the first syllable to the end of the terminal syllable of each respective song (Boogert et al. 2008). It can also be considered a temporal song variable (Turčoková et al. 2010) and has been shown to be positively correlated with the volume of a telencephalic song system nucleus, the HVC (Eens et al. 1991), which controls the learning and production of vocal signals in songbirds (Gil & Gahr 2002; Buchanan et al. 2004). Syllable diversity is defined by the number of different syllables in an individual's song (Garamszegi et al. 2003; Boogert et al. 2008; Yu et al. 2017). Different syllables were identified and distinguished by their duration, spectral structure, and bandwidth (the difference between minimum and maximum frequencies), Females favour a large diversity of syllables when selecting mates (Woodgate et al. 2011). Syllable rate is a calculation of the number of syllables produced per second (Feßl & Hoi 2000). In addition to a song complexity measure, it can also be used as a measure of song performance (DuBois et al. 2009; reviewed in Linhart et al. 2013) and can reflect male quality (Ballentine et al. 2004). Evidence intimates that females may also select males with a high number of syllable transitions, specifically a high degree of switching from one syllable type to another (Woodgate et al. 2011). The total number of syllables per song differs discreetly from syllable diversity in that it is simply an overall tally of all syllables within a particular song (Boogert et al. 2008). Finally, in those species that produce trills, females perceive them as predictors of male quality; such are the high energy demands of producing trill components (Ballentine et al. 2004; Hennin et al. 2009; Kagawa & Soma 2013). Trills have been shown to be aggressive signals in territorial male nightingales (Schmidt et al. 2008) and swamp sparrows (DuBois et al. 2009). These within-song complexity variables can be analysed, extracted and compared using song spectrograms across different individuals and species.

1.2 Main study species

Tui are medium-sized honeyeaters endemic to New Zealand. Though nectarivorous, they also eat fruits, seeds and arthropods (Craig et al. 1981; Bergquist 1985). Tui inhabit mature mixed podocarp forests, but also coastal forests of pohutukawa (*Metrosideros excelsa*), kowhai (*Sophora microphylla*) and rewarewa (*Knightia excelsa*) as well as regeneration forests of puriri (*Vitex lucens*). Tui are also found in suburban gardens with introduced flowering plants (Bergquist 1989), and are important pollinators of native New Zealand plants such as those mentioned above in addition to species such as flax (*Phormium tenax*), kohekohe (*Dysoxylum spectabile*) and fivefinger (*Pseudopanax arboreus*). The effective dispersal of small fruits of most plant species within the New Zealand forest relies heavily on tui (Burns & Lake 2009; Kelly et al. 2010).

Little was known about much of the behavioural ecology of tui until comparatively recently. Recent research however suggests tui have extreme sexual dimorphism in addition to a high EPP frequency (Wells et al. 2015). Within one study population, 57% of all tui offspring were fathered by EP males (Wells et al. 2015). Similarly, tui song has not been researched extensively until recently. Evidence suggests that tui can produce up to 56 different syllables within a song (Hill et al. 2013), and hundreds of syllables within a repertoire. Tui also produce many different song types, both broadcast and interactive (Hill 2011). Complex songs are used in dominance hierarchy resolutions (Bergquist & Craig 1988; Bergquist 1989), and in aggressive territoriality (Hill et al. 2013). Furthermore, recent studies suggested mainland tui produce more complex songs than those on remote islands (Hill et al. 2013). Another recent study indicated tui song frequency and complexity varies within urban habitats. Specifically, individuals inhabiting areas close to motorway zones produce songs with higher minimum frequency and increased syllable diversity (Ludbrook 2015). Recent evidence therefore indicates the presence of intraspecific tui song variation, nevertheless much remains unknown about tui vocal behaviour and factors that are associated with its variation. Investigating variation in tui song complexity between individuals and populations contributes important insights into the behavioural ecology of an endemic honeyeater species critical to New Zealand’s ecology.

The high song complexity levels observed in tui are comparable to those of other, more commonly studied songbird species with highly complex vocalisations, such as the common nightingale, skylark (*Alauda arvensis*) and winter wren (Figure 1.1; Table 1.1). Tui therefore represent a suitable model for examining the ecological, evolutionary and behavioural theory of song complexity variation in songbirds. One major advantage of examining the vocal behaviour within vocally complex species is that it can provide important insights into the importance of multiple acoustic cues for female mating choice (Bartsch et al. 2015).

Table 1.1: An overview of the vocal behaviour of tui and ten other selected songbird species described in the literature as having highly complex vocalisations.

Songbird species	**Common name**	**Song complexity characteristic(s)**	**Literature references**
Prosthemadera novaeseelandiae	Tui	Mean syllable diversity per song: 23	Hill et al. 2013;
		Max syllable diversity per song: 56	Hill & Ji 2014
		Song type repertoire: >30	
Alauda arvensis	Skylark	Mean syllable repertoire: 30	Briefer et al. 2008
Emberiza tahapisi	Cinnamon-breasted bunting	Mean syllable diversity per song: 7	Osiejuk 2011
		Max syllable diversity per song: 9	
Luscinia megarhynchos	Common nightingale	Syllable repertoire: 1,166	Kiefer et al. 2009;
		Song repertoire: 190	Moore et al. 2011;
			Bartsch et al. 2015
Menura novaehollandiae	Superb lyrebird	Max syllable diversity per song: 8	Zann & Dunstan 2008;
		Song types: 90	Dalziell et al. 2012
Passerella iliaca	Fox sparrow	Mean syllable diversity per song: 8	Miller 1982;
		Max syllable diversity per song: 11	Naugler & Smith 1991
		Mean song type repertoire: 2	
		Max song type repertoire: 3	
Sturnus vulgaris	European starling	Mean motifs (sequences of syllables) per song: 70	Hultsch & Todt 2004
		Max motifs per song: 80	
Toxostoma redivivum	California thrasher	Max syllable diversity per song: 35	Sasahara et al. 2012;
			Cody et al. 2016
Toxostoma rufum	Brown thrasher	Max syllable diversity per song bout: 5	Boughey & Thompson 1981;
		Song type repertoire: 1,147	Kroodsma 2005
Troglodytes hiemalis	Winter wren	Max syllable diversity per song: 40	Kroodsma 1981
		Syllable repertoire:100	
Vireo olivaceus	Red-eyed vireo	Max syllable diversity per song: 6	Borror 1981;
		Mean song type repertoire: 39	van Roo et al. 2004

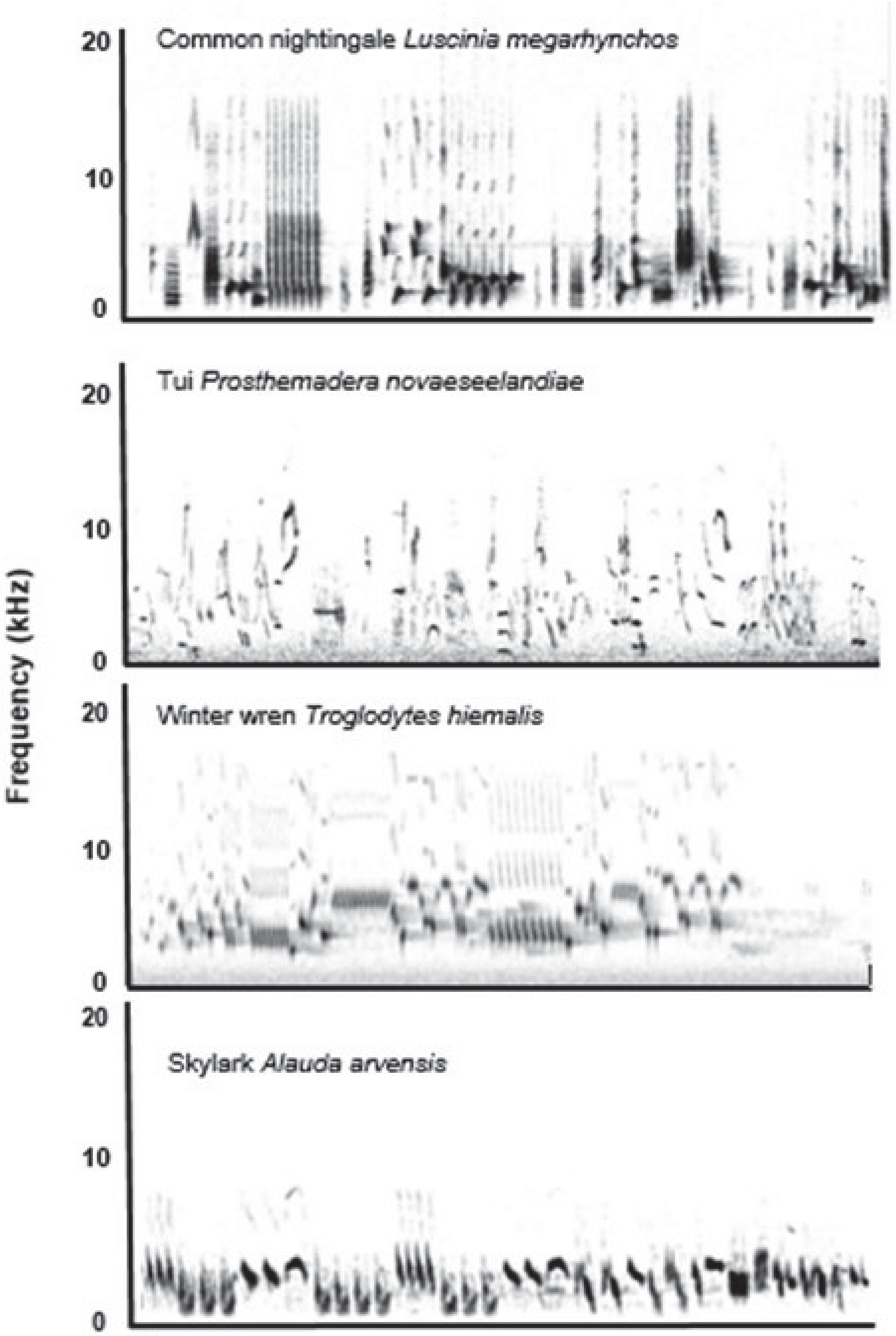


Figure 1.2: Ten-second song spectrographic examples of tui and three other vocally complex songbird species. The frame length was set at 256 points, 50 % frame overlap with a hop size of 2.9 milliseconds was used. Frequency grid spacing of 172 Hz was also employed and the bandwidth was set at 3 dB.

1.3 Thesis structure and objectives

The main aim of this thesis was to explore a series of key biological and environmental factors and their correlative association with song complexity to enhance our knowledge and understanding of songbird song complexity evolution. As discussed, the species used within this thesis was tui, a good model for the exploration of song complexity variation, as it has highly complex vocalisations, and thus has the potential for large song and syllable variation. Using tui as the main model, I examined the effects of a series of biological and environmental factors on songbird song complexity with observational and experimental approaches in order to answer the following research questions:

1. Is song complexity important in the evolution of extra-pair paternity (EPP) in a socially monogamous songbird? Selection pressures generally favour the evolution of sexually-selected characteristics reflecting male quality (Purves et al. 2016) such as song complexity (e.g. Garamszegi & Møller 2004). This is particularly evident in species with high EPP frequencies, as these males usually do not provide parental care (Møller 2000). In addition, it was predicted that there would be a positive association between song complexity and extra-pair paternity frequency, 2. In species with high EPP frequencies, is song complexity a more important trait for broadcast songs, than in interactive songs? Broadcast songs are generally intended for distant audiences, therefore, likely play an important role in attracting both social and EP mates. On the other hand, interactive songs are generally directed at close

range towards mates or intruders. Interactive songs are thus often used in male-male confrontations (Titus 1998), and courtship and pair-bond maintenance (Titus 1998; Reichard et al. 2011). Broadcast songs should therefore contain high complexity in species with high EPP frequencies if song complexity is selected in EP mating. Therefore, song complexity was predicted to be higher in broadcast than in interactive songs, 3. Does song complexity vary at different times of day, and what factors may influence this variation? Multiple vocal traits have been shown to be intensified or increased at dawn including song output (Otter et al. 1997; Poesel et al. 2001; Otter & Ratcliffe 2005; Catchpole & Slater 2008; Erne & Amrhein 2008; Sandoval & Mennill 2014; Quispe et al. 2016). Therefore, it was predicted that tui song complexity would exhibit the same pattern and be increased at dawn. These increases in vocal intensity may reduce intrusion rate in songbirds (Yasukawa 1981; De Kort et al. 2009), therefore increases in dawn tui song complexity exhibiting associations with reduced intrusion rates was also predicted, 4. Does song complexity indicate the competitiveness of a male and do territorial males respond more strongly to unfamiliar songs with higher complexity? Since songbird song complexity plays a role in territory tenure, and female extra-pair mate selection (Potvin et al. 2015), intruding males with more complex songs are likely to be perceived as stronger competitors by territorial males. Hypothetically, this would be especially true in species with high extra-pair paternity who risk suffering mate cuckoldry and territory loss. Therefore, it was predicted that complex songs of intruding male tui would indicate a greater challenge to territorial males, and thus would elicit stronger, more aggressive responses than simpler songs, 5. Does habitat complexity have an association with songbird song complexity? Variation in song complexity traits may be generated by acoustic adaptation to divergent habitat types

(Handford & Nottebohm 1978; Tubaro et al. 1993). Previous evidence suggests that trills and songs are longer in closed and more complex habitats (Wiley 1991; Van Dongen & Mulder 2006). However, there is currently no strong evidence that other song complexity characteristics such as syllable diversity and entropy vary in divergent habitats. It was predicted that these traits would also be higher in more complex habitats. Each research question was addressed per chapter. This thesis comprises four data chapters (Chapters 2 to 5), with an introductory (Chapter 1), and an overall summary chapter which includes future directions (Chapter 6). The data chapters have been written as papers for peer-reviewed publication.

Chapter 1 provides a background to songbird vocalisation studies, song complexity and identifies knowledge gaps and how I intended to address these gaps with my PhD research. I also describe the main study species, the tui, and why it was chosen as a study subject to explore song complexity variation. Also included in this chapter are the general methods employed in this thesis to measure song complexity.

Chapter 2 explores variation in song complexity using 78 songbird species and its association with EPP frequency. To establish clarity and further understanding of the biological significance of associations between EPP and song complexity, advancing from previous meta-analyses, the following aspects were considered and implemented: 1. conducting spectrographic song analyses from recordings thereby promoting analytical consistency in multiple species, 2. increasing the number of songbird species that were sampled by Garamszegi & Møller (2004) and 3. comparing song complexity between broadcast songs (likely produced in part for mate attraction) and interactive songs in a high EPP frequency species, the tui. Furthermore, Chapter 2 examines the variation in song complexity of two different song classes (broadcast and interactive) within a species with high EPP frequency. The findings suggest that song complexity may have evolved under EPP mate selection pressures. By investigating variation in song complexity across a multitude of species and exploring its relationship with EPP, this chapter enhances our understanding of song complexity evolution across taxa. This chapter was submitted and accepted for publication: Hill SD, Amiot C, Anderson MG, Ji W. 2017. It's complicated: association between songbird extra-pair paternity and within-song complexity. Animal Behaviour 130: 187–197.

Chapter 3 examines the diurnal variation in tui song complexity and territorial intrusions. It also investigates associations between song complexity and intruder pressure/intrusion rates. This chapter provides novel findings in a songbird species and contributes knowledge into song complexity evolution within a territoriality framework. This chapter was submitted and accepted for publication: Hill SD, Pawley MDM, Anderson MG, Ji W. 2017. Higher song complexity and intrusion rate at dawn in a vocally complex songbird. Emu 118(1): 1–11.

Chapter 4 focuses on the function of complex song in species with repertoires of differing complexity levels. This was done by assessing the responses of territorial male tui to differing levels of tui song complexity via playback experiments. Male responses were measured in terms of their levels of aggression using nine variables including the song complexity response of territorial males. This chapter was submitted and accepted for publication: Hill SD, Anderson MG, Brunton DH, Ji W. 2017. Fighting talk: complex song elicits more aggressive responses in a vocally complex songbird. Ibis (doi: 10.1111/ibi.12542).

Chapter 5 investigates the variation in tui song complexity across divergent habitat types (closed forest habitats and open habitats). This was conducted by comparing the song complexity levels of tui within two separate regional parks. It provides important insights into the potential drivers of complex song evolution in this species. Additionally, it presents novel findings within a songbird species. This chapter was submitted and accepted for publication: Hill SD, Pawley MDM, Ji W. 2017. Local habitat complexity correlates with song complexity in a vocally elaborate honeyeater. Austral Ecology 42(5): 590–596.

Chapter 6 is a general conclusions chapter providing an overall summary within this research, and how these findings fit into the general framework of song complexity variation in songbirds. I also outline the novel contribution this thesis has provided in this field of research. In addition, I describe the future directions that have been identified from the research within this thesis.

Table 1.2: A summary of the research gaps, and the research questions designed to help address these research gaps within the four data chapters of this thesis.

	Research gap	Main research question(s)	Chapter title
1.	A) It is unclear why some songbird species have evolved high song complexity and others lower. B) It is not known whether broadcast songs in high extra-pair paternity species are more complex than interactive songs	Is there a relationship between extra-pair paternity frequency and song complexity in songbirds? Are the broadcast songs of a high extra-pair paternity species more complex than interactive songs?	It's complicated: the association between songbird extra-pair paternity and within-song complexity
2.	It is uncertain whether both songbird song complexity and the number of attempted territorial invasions vary during the diurnal period. It is unclear also whether song complexity has an association with the number of agonistic encounters or territorial invasions (i.e. intruder pressure)	Do song complexity and intrusion rates vary throughout the diurnal period? Is there a relationship between song complexity and the number of territorial invasions (i.e. intruder pressure) in a vocally complex and highly territorial songbird?	Higher song complexity and intruder pressure at dawn in a vocally complex songbird
3.	Few studies have examined highly vocally complex species that produce songs with differing levels of complexity within their repertoire. Furthermore, few studies have attempted to ascertain the function of songs with different levels of complexity	What are the responses of territorial males to male songs with differing levels of complexity?	Fighting talk: complex song elicits more aggressive responses in a vocally complex songbird
4.	Differing degrees of song complexity at the microgeographic level in areas of differing habitat complexity has not been investigated	Does habitat complexity correlate with song complexity in tui?	Local habitat complexity correlates with song complexity in a vocally elaborate honeyeater

2 It’s complicated: the association between songbird extra-pair paternity and within-song complexity

Adult tui with fledgling (photo: Anna Arrol, with permission).

This chapter was published in *Animal Behaviour* (Appendix 1).

Abstract

Many songbird species are socially monogamous whilst exhibiting varying extra-pair paternity frequencies. Song complexity, often subject to sexual selection, similarly varies across songbird taxa. Some species form highly complex songs whereas others produce simple songs. The basis of this variability however is unresolved. Because selection pressures generally favour the evolution of sexually-selected characteristics reflecting male quality, such as song complexity, it should be subject to extra-pair mate selection. I therefore predicted a positive association between extra-pair paternity frequency and songbird song complexity. In addition, I predicted that broadcast (long-range) rather than interactive songs (short-range) would be more likely to contain sexually-selected characteristics, such as higher complexity, especially in species with high extra-pair paternity frequencies. This was tested using tui (*Prosthemadera novaeseelandiae*), a species with high extra-pair paternity frequency. Firstly, analyses on 78 songbird species indicated a significant positive association between extra-pair paternity frequency and within-song complexity (the level of complexity within a species-specific song) but not between-song complexity (size of species-specific song or syllable repertoire), whilst no phylogenetic trait conservation was found. Additionally, the results suggested tui broadcast songs had higher song complexity than interactive songs. The findings in this study indicate extra-pair mate selection may play a role in the evolution of within-song complexity in songbirds.

Keywords: broadcast song, extra-pair paternity frequency, interactive song, *Prosthemadera novaeseelandiae,* song complexity, songbirds, tui

2.1 Introduction

Bird song is a learned behaviour and, in some songbirds, complex song is a sexually-selected trait (Catchpole & Slater 2008). Song functions predominantly for mate attraction and territory defence (Andersson 1994; Searcy & Yasukawa 1996; Leniowski & Wegrzyn 2013), and male songs may be assessed by both females and rival males (Illes et al. 2006). Evidence suggests unpaired males will sing long, complex songs; in contrast, paired males produce shorter, simpler songs (Catchpole 1983). Females produce more copulatory displays to males with complex songs (Catchpole et al. 1986). Furthermore, males with more complex songs will be paired earlier within the breeding season, attract larger numbers of females and produce more young (Catchpole 1986; Catchpole 1992). Male song also functions to deter rivals from entering territories and copulating with their social partners during the female fertile period (Catchpole & Slater 2008).

Song complexity can be measured by calculating the total song or syllable repertoire size and song versatility of individuals (Hasselquist et al. 1996; Garamszegi & Møller 2004; Moore et al. 2011), termed ‘between-song complexity’ (Ballentine et al. 2003). Alternatively, song complexity can be measured using variables such as the mean number of different syllables per song and the number of transitions between different syllable types (Leitão et al. 2006; Sasahara et al. 2012). Song complexity is likely to be a proxy for male quality in terms of reproductive success, body condition, longevity, low developmental stress, parental care and cognitive ability (Buchanan & Catchpole 2000; Badyaev et al. 2002; Spencer et al. 2005; Sexton et al. 2007; Boogert et al. 2008; Catchpole & Slater 2008; Woodgate et al. 2011, 2012; Soma & Garamszegi 2011). Degrees of song complexity vary widely across-taxa, from simple, monotonous patterns composed of a few syllables (e.g. zebra finches *Taeniopygia guttata*, Woolley & Doupe 2008 and European warblers Genus: *Acrocephalus*, Catchpole 1980) to highly complex songs with a large syllable diversity, organised in non-random fashion with discernible patterns (e.g. European starlings *Sturnus vulgaris*, Moorman & Bolhuis 2013 and California thrasher *Toxostoma redivivum* Sasahara et al. 2012). Notwithstanding, song complexity variation occurs *within* taxonomic families. In the honeyeaters (Meliphagidae) for example, the repertoires of noisy miners (*Manorina melanocephala*) and New Holland honeyeaters (*Phylidonyris novaehollandiae*) consist of simple single high-frequency notes (Jurisevic & Sanderson 1994). Tui (*Prosthemadera novaeseelandiae*) on the other hand sing highly complex songs consisting of many different syllables (Hill 2011; Hill et al. 2015). The evolutionary basis of phenotypic songbird variation in song complexity levels is somewhat of a conundrum to evolutionary ecologists (Kaluthota 2016).

Extra-pair paternity (EPP) can be defined as ‘deviations from genetic monogamy’ (Turjeman et al. 2016), where males sire offspring outside their social bond (e.g. Forstmeier et al. 2014). In songbirds, it is often directed by a female who will initiate EP mating (Bowers et al. 2015). Extra-pair paternity is widespread across songbird taxa (e.g. Taylor et al. 2008) despite many species being socially monogamous (Hasselquist & Sherman 2001; Liu et al. 2015). Extra-pair copulations are more likely to occur in species where bi-parental care is of low importance to chick survival (Petrie & Kempenaers 1998). Females may attain direct benefits from EP copulations. For example, they may be able to feed on an EP partner's territory, in addition to attaining lower depredation and starvation rates in EP offspring (Gray 1997). Moreover, EP mating enhances the inclusive fitness of females beyond the genetic capability of their social partner (Bowers et al. 2015). This is reflected in the increased additive genetic value of offspring, often referred to as the 'good genes hypothesis' (see Reid et al. 2011). Offspring of EP males may also have greater heterozygosity (Foerster et al. 2003).

Extra-pair paternity frequency varies within- (Spottiswood & Møller 2004) and across-taxa (Canal et al. 2012). For example, EPP frequency in the Savi's warbler (*Locustella luscinioides*) is 4.1% of all offspring observed (Neto et al. 2010) whereas in superb fairy-wrens 65% of offspring are as a result of EPP (*Malurus splendens*, Tarvin et al. 2005). Furthermore, EPP frequency in the great reed warbler (*Acrocephalus arundinaceus*) is 4% (Hasselquist et al. 1995) and in a tui population, 57% of offspring observed resulted from EPP (Wells et al 2015).

Selection pressures generally favour the evolution of sexually-selected characteristics reflecting male genetic quality (e.g. Kempenaers et al. 1992; Garamszegi & Møller 2004). This is particularly evident in species with high EPP frequencies, as these males usually do not provide parental care (Møller 2000). We would therefore predict that song complexity would be more pronounced in species with high EPP frequencies, as males of these species would be under more intense sexual selection pressures. In other words, there would be a significant positive association between EPP frequency and song complexity across taxa. Some evidence supports this prediction and indeed females have been found to select EP males with larger song repertoires and higher levels of song consistency (Read & Weary 1992; Hasselquist et al. 1995; Byers 2007). However, other studies including a meta-analysis using 65 songbird species (Garamszegi & Møller 2004, Soma & Garamszegi 2011) found no clear relationship between EPP and multiple song features. Therefore, whether male song traits play a role in EPP mate selection remains uncertain. To test the prediction that EPP frequency was positively associated with song complexity in multiple songbird species, I employed both within-and between-song complexity measures in addition to a larger sample size of 78 species. Advancing from the above-mentioned meta-analyses, I also extracted and analysed song complexity data from song recordings both from online databases, and from field recordings of tui and yellowhammer (*Emberiza citrinella*).

Songbird songs have been categorised into two classes according to audience and transmission range: long-range broadcast songs (BS) and short-range interactive songs, IS (Titus 1998; Nolan et al. 2002; Rosvall et al. 2012; Reichard et al. 2013). Broadcast songs are defined as prominent and non-directional vocal signals, functioning as advertisements of male reproductive and territorial status (Wiley & Richards 1982; Titus 1998). They are directed to no obvious audience; therefore, likely play an important role in attracting both social and EP mates. Interactive songs on the other hand are often initiated and directed towards an observable audience. They are employed in male-male confrontations (Titus 1998), in addition to courtship and pair-bond maintenance (Titus 1998; Reichard et al. 2011). If song complexity is selected in EP mating, BS should contain high complexity in species with high EPP frequencies. Little information is available regarding differences in song classes in the context of EPP mate selection.

Previous research suggested short-range songs (IS) in the dark-eyed junco (*Junco hyemalis*), a species with moderate EPP frequency (27%, Gerlach et al. 2012), are more complex than long-range songs (BS) in terms of syllable diversity. Using tui, a species with both very high EPP frequency and song complexity, I investigated the role of BS within the context of mate attraction and examine whether BS differentiate in complexity to IS. I predicted that BS would contain higher levels of within-song complexity than IS.

2.2 Methods

2.2.1 Data collection: EPP and song complexity in multiple species

Male, breeding season songs of 78 songbird species were collected; 76 of which were obtained exclusively from online databases, Macaulay Library archive of wildlife sounds and videos of Cornell Lab of Ornithology (Cornell University; www.macaulaylibrary.org) and the Xeno-Canto citizen science project website (www.xeno-canto.org). Songs were extracted from 180 recording files from the Macaulay Library and 52 from Xeno-Canto. Xeno-Canto only supports mp3 files and these were converted to wav files using http://www.audio.online-convert.com/convert-to-wav. The Xeno-Canto archive has been used to provide compressed sound files in other studies analysing and examining avian song structure (Weir & Wheatcroft 2011) without having any significant impact on song structural integrity. However, I acknowledge that the recording quality on these files may have been marginally lower than for the other sound samples used in this study. This is however unlikely to have had any significant effect on analyses within this study. In addition, recordings of tui and an opportunistic recording of a single yellowhammer were obtained in the field at Tawharanui Regional Park, New Zealand, using a Marantz PMD620 Solid-State Digital Recorder (Marantz, Kanagawa, Japan), attached to a Sennheiser ME67 shotgun long-range directional microphone (Sennheiser, Old Lyme, CT). All songs were recorded at a sampling frequency of 44.1 kHz and at a resolution of 16-bits. The frequency response range of the long-range microphone was 40 to 20 000 Hz.

All species analysed within this study included 63 of the 65 analysed in a previous metaanalysis by Garamszegi & Møller (2004). This study excluded wood warbler (*Phylloscopus sibilatrix*) and canary (*Serinus canaria*) due to these species exhibiting zero EPP. The remaining 15 species were chosen to obtain a wider range of EPP frequency and to increase sample size from this previous study. Multiple species within this dataset have socially monogamous mating systems with varying EPP frequencies (see Appendix 2). The EPP frequency data for each species were obtained from highly cited peer-reviewed research articles found in the Google Scholar (Mountainview, CA, USA; www.scholar.google.com) and Web of Science (Clarivate Analytics, USA; www.clarivate.com/?product=web-of-science) databases, in addition to research articles utilised by Garamszegi & Møller (2004) and Soma & Garamszegi (2011). Some published articles were the only research papers available on specific species. Some species within this dataset have also been noted as exhibiting polygamous (a male or female having more than one social partner, Hasselquist et al. 1995) or cooperative breeding behaviour (exhibition of alloparental offspring care, where parental care of young is augmented by other group members, Piper & Slater 1993).

When songs from multiple individuals of the same species were available, a single randomly-selected song was chosen from each of three randomly selected individuals (aside from one species, Henderson reed warbler *Acrocephalus taiti*, where only one male recording was available) was analysed (totalling 232 individuals). I checked every song within each respective individual's recording and ensured that the song analysed was of comparable levels of complexity to the rest of the songs within its repertoire on that recording, in terms of number of syllables, song duration and syllable diversity. In species with smaller song repertoires, the songs used for analysis were often repeated within a recording. Only recordings with the highest quality rating in each database were used (i.e. those with a good signal-to-noise ratio). The time, date, location, context, whether songs were natural or induced using playback (all songs used in analyses were natural), habitat (I maintained intraspecific habitat consistency in the choice of recordings where possible i.e. recordings from each respective species were in similar habitat types) and comprehensive details of each song in terms of their song class (i.e. BS or IS) were available and extracted from the database. Only BS were used in this part of the study to avoid any confounding factors because differences in BS and IS structure and complexity have been noted as described. Furthermore, the most common song types available were BS. Analysis of the differences in complexity between BS and IS was conducted in a high EPP species, tui (details later within this section).

2.2.2 Data collection: variation between tui song classes

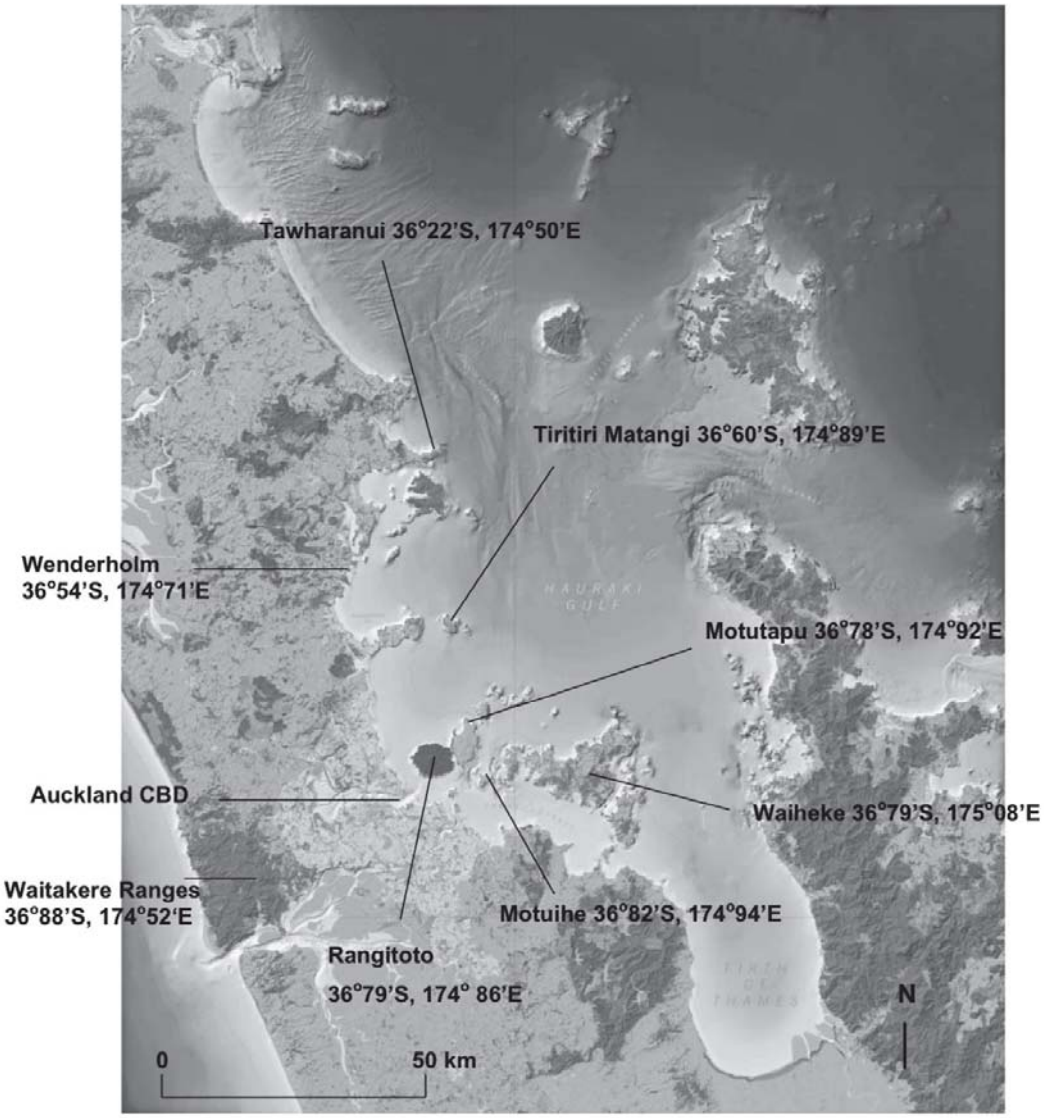
Tui are endemic New Zealand songbirds within the family Meliphagidae (Robertson et al. 2011). They are socially monogamous with high EPP frequency (57%, Wells et al. 2015), high sexual size dimorphism (Wells et al. 2015), and exhibit highly agonistic behaviour (Craig 1984). Tui have high song complexity and a large song and syllable repertoire (Hill & Ji 2014), likely to be products of sexual selection.

Song data from adult male tui were collected from 11 different rural locations across New Zealand's North Island and nearby offshore islands (Figure 2.1a, 2.1b, Table 2.1). Data collection was carried out over four breeding seasons (October-February 2010/2011, 2011/2012, 2012/2013, and 2013/2014). During sampling sessions, we walked slowly along marked tracks to locate tui at all recording sites. When a tui was located, recording was initiated and ended when it either ceased singing or departed its singing perch (the total sampling effort per individual varied (minimum: 1.8 min, maximum: 12.9 min). Recording however was reinitiated if the same individual started singing again within 5 min of cessation. During each recording, the location, time, the band combination of the tui (if applicable), behaviour, and sex were noted. Broadcast songs were identified as such when tui projected songs from high vantage points to no immediately observable audience. Interactive songs were identified as such when directed, at close-range (between 0-2 m), towards another individual, either male or female.

Among the 28 males recorded at Tawharanui Regional Park, 17 were individually marked with colour bands. For each banded bird, I only analysed songs that were recorded in a single three-hour session. For unbanded birds, only one unbanded individual was recorded at any one time and in any one identified territory across locations to minimise the chances of pseudoreplication. During the breeding season when tui are paired and have nests, both nests and flowering plants are guarded, and tui remain within their respective territories. Furthermore, males also tend to sing on specific perches (Hill et al. 2013) which further minimised the chances of pseudoreplication. All songs included in the analysis were recorded between 0600 and 1500 hours at between 2 m and 5 m from each bird. Only complete songs with good signal-to-noise ratios were included in analyses.

Four variables measuring song complexity were extracted from each song: number of syllables, song duration, syllable diversity, and syllable transitions (Table 2.1) using Raven Pro. An overall song complexity score was then created by adding together the values for each of the four complexity variables for each individual (adapted from the methodology described by Ulubay 2013). I analysed songs from 180 recording files from Macaulay Library and 52 from Xeno-Canto. For all recordings, spectrograms were created by Discrete Fourier Transform (DFT) with a Hann window. The frame length was set at 256 points. Additionally, a 50 % frame overlap with a hop size of 2.9 milliseconds was used. Frequency grid spacing of 172 Hz was also employed and the bandwidth was set at 3 dB.

(a)


Tawharanui 36°22'S, 174°50'E
Tiritiri Matangi 36°60'S, 174°89'E
Wenderholm
36°54'S, 174°71'E
HAURAKI GULF
Motutapu 36°78'S, 174°92'E
Auckland CBD
Waiheke 36°79'S, 175°08'E
Waitakere Ranges
36°88'S, 174°52'E
Motuihe 36°82'S, 174°94'E
Rangitoto
36°79'S, 174° 86'E
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(b)

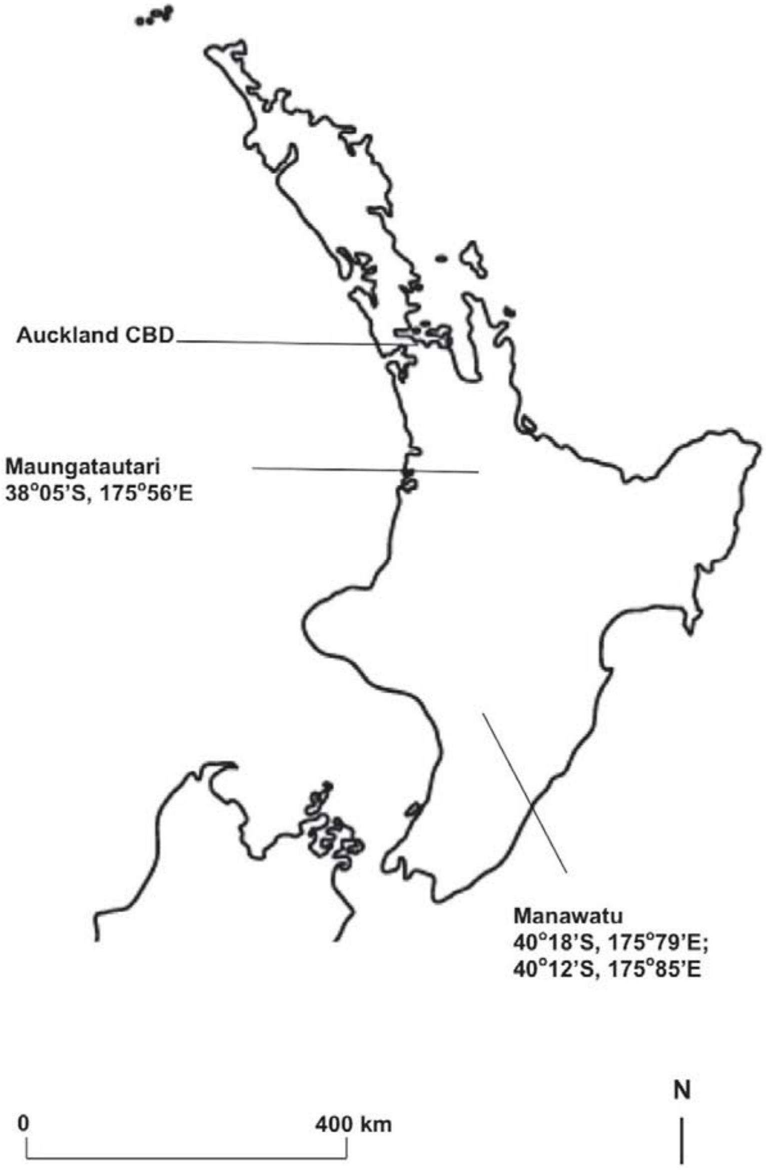


Figure 2.1: The location of the eight study sites around the Auckland region including a) the five offshore islands (map from NIWA, with permission) and b) the location of the three mainland study sites in relation to Auckland's central business district.

Table 2.1: Tui song recording sites and number of tui recorded.

Location	Size (ha)	Endemic songbird species present	Number of tui sampled
Ark in the Park, Waitakere	1100	*Callaeas wilsoni, Gerygone igata, Petroica longipes, Petroica macrocephala toitoi, Rhipidura fuliginosa,*	3
Maungatautari southern Enclosure	65	*Anthornis melanura, Gerygone igata, Mohoua albicilla, Notiomystis cyncta, Petroica longipes, Petroica macrocephela toitoi, Philesturnus rufusater, Rhipidura fuliginosa*	3
Motuihe	179	*Gerygone igata, Mohoua albicilla, Philesturnus rufusater, Rhipidura fuliginosa*	2
Motutapu	1510	*Anthornis melanura, Gerygone igata, Philesturnus rufusater, Rhipidura fuliginosa*	1
Pohangina wetlands	7.2	*Anthornis melanura, Gerygone igata, Rhipidura fuliginosa*	1
Rangitoto	2311	*Anthornis melanura, Gerygone igata, Mohoua albicilla, Petroica macrocephela toitoi, Philesturnus rufusater, Rhipidura fuliginosa*	2
Tawharanui Regional Park	588	*Anthornis melanura, Gerygone igata Mohoua albicilla, Petroica longipes Philesturnus rufusater*	28
Tiritiri Matangi	254	*Acanthisitta chloris*, *Anthornis melanura*, *Calleaus wilsoni, Gerygone igata, Mohoua albicilla*, *Petroica longipes*, *Philesturnus rufusater*, *Rhipidura fuliginosa*	3
Totara Reserve, Pohangina	340	*Anthornis melanura, Gerygone igata, Rhipidura fuliginosa*	1
Victoria Reserve, Waiheke	11	*Gerygone igata, Rhipidura fuliginosa*	3
Wenderholm Regional Park	134	*Gerygone igata, Rhipidura fuliginosa*	4

2.2.3 Song variables and data analysis for EPP and song complexity in multiple species

To assess the relationship between EPP frequency and both within- and between-song complexity linear regressions were employed (SPSS, version 23.0; IBM, Chicago, IL, USA). Four variables measuring within-song complexity were extracted from each song: number of syllables, song duration, syllable diversity, and syllable transitions (Table 2.2) using Raven Pro. Evidence suggests these four song complexity variables within male BS are assessed and sexually selected by females therefore are important in mate attraction (Boogert et al. 2008). An overall within-song complexity score was then created by adding together the values for each of these four complexity variables for each individual (adapted from the methodology described by Ulubay 2013). All song complexity scores were log-transformed prior to linear regression analyses as they were non-parametric in distribution, as determined by Kolmogorov-Smirnov tests (using SPSS).

I also extracted three key between-song complexity variables, song and syllable repertoire size in addition to song versatility from the same recordings, as above to maintain consistency (Table 2.2). Song and syllable repertoire size were quantified by tallying the number of unique songs and syllables produced by each individual in each total recording. The number of songs however varied in each recording therefore when measuring between-song complexity the song repertoire size score for each individual was based upon the percentage of songs recorded that were unique within that individuals' repertoire (i.e. the number of unique song types produced by an individual was divided by the total number of songs recorded for that individual and then that number was multiplied by 100 [adapted from Podos et al. 1992]). To measure song versatility, songs were scored as being versatile (score of two) if song types were switched rapidly (for example if song type 1 was immediately followed by song type 2, then immediately followed by song type 3 etc); eventually versatile (score of one), if song types were repeated within a repertoire before eventually switching; and non-versatile (score of zero) if a song type was repeated continuously within a repertoire (as described by Garamszegi & Møller 2004). House sparrow *Passer domesticus* was excluded from the between-song complexity analysis as their songs only consisted of one syllable and distinguishing between songs was not possible, and Henderson reed warbler, for which only one recorded song was available for analysis. All song values used within this part of the study were averaged for each species for all statistical analyses.

Songs from each species were analysed blind to their respective EPP frequencies to avoid potential sampling bias. Recordings (written as wav files at a sampling rate of 44.1 kHz and at a resolution of 16-bits) were digitised and song variables measured using Raven Pro 1.4 Beta Version software (Cornell Lab of Ornithology, Ithaca, NY, USA). A song is defined as a period of continuous syllables surrounded, before and after, by a silent inter-song interval period of ≥ 3 seconds (Woolley & Rubel 1997) and only complete songs were analysed. All 78 species analysed produced discontinuous songs, or songs that have discernible inter-song intervals.

Table 2.2: Song variables employed in this study to compare song complexity in 78 different songbird species with varying EPP frequencies, and between BS and IS in tui.

Song variable	Definition
Number of syllables	The total number of sound units or sound unit complexes (syllables) per song. A note alone without a repeat was considered a syllable. When multiple identical notes were repeated consecutively, this cluster was also considered a single syllable (Boogert et al. 2008)
Song duration (seconds)	The length of time from the start of a note or syllable to the end of a note or syllable in a series of syllables preceded and followed by a ≥ 3 second interval (Kroodsma 1977)
Syllable diversity	The number of different sound units or sound unit complexes (syllables) per song. Syllables can be comprised of several notes (Boogert et al. 2008)
Syllable transitions (or versatility)	The number of syllable switches per song (Feßl & Hoi 2000)
Trill duration (seconds)	Cumulative total length of repeated, high-frequency modulation portions per song (Hill et al. 2015)
Trill percentage	Percentage of each song that contains rapid, repeated, high-frequency modulation notes. Computed by dividing song duration with the amount of time (seconds) trills are produced within a song (Hill et al. 2013).

2.2.4 Phylogenetic correction

Comparative analyses examining ecological differences across taxa need to consider potential effects due to shared ancestry (Lifjeld et al. 2010). A phylogenetic tree for these 78 species was created using ‘a global phylogeny of birds’ (available from: http://birdtree.org) based on the most comprehensive studies (Jetz et al. 2012, 2014). An image of the phylogenetic tree from the resulting file was created using FigTree (version 1.4.2, Figure 2.2). The branch length units are based on nucleotide substitutions per site i.e. the number of changes or 'substitutions' divided by the length of the sequence. We used phylogenetic generalised least squares (PGLS) for phylogenetic correction to determine whether phylogeny significantly influenced a linear regression analysis (as per Barger et al. 2014, conducted in SPSS) testing for associations between EPP frequency and song complexity. The PGLS estimates the value of lambda (λ, Pagel 1999) to estimate phylogenetic correlation. If λ = ‘1’, the diversification ratio is phylogenetically conserved; conversely, if λ = ‘0’ this implies the diversification ratio is random with respect to phylogeny (i.e. the traits are independent of phylogeny, in other words, there is more divergence between taxa than expected under a Brownian motion model of trait evolution). The PGLS analysis was carried out using the nlme package (Pinheiro et al. 2013) in R (version 2.15.3). For model selection, we used the second-order Akaike Information Criterion (AIC, Burnham & Anderson 2002) and likelihood ratio (LR) tests. The PGLS estimated λ for the model and tested whether it was significantly different from zero. All song values were averaged for each species.

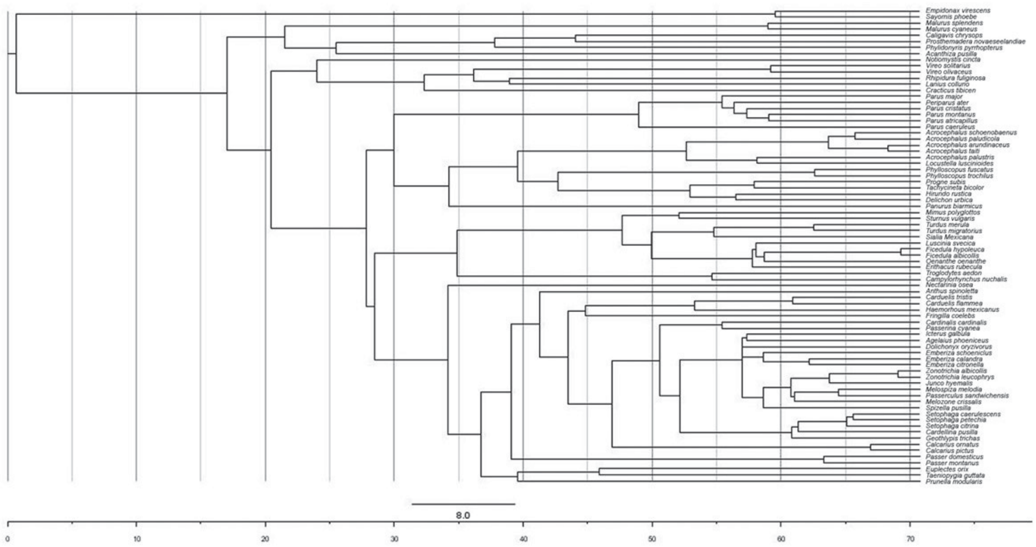


Figure 2.2: The phylogenetic tree of the 78 songbird species within this study, including the scale. The units of branch length are nucleotide substitutions per site i.e. the number of changes or 'substitutions' divided by the length of the sequence.

2.2.5 Mating system

Mating system is associated with sperm competition in songbirds, which is widespread in socially monogamous species that undertake extra-pair copulations (Birkhead 1998). I therefore controlled for mating system in the comparative analyses of the 78 songbird species. To test whether the different mating systems described above (categorised as: socially monogamous (1), polygamous (2), or cooperative (3) extracted from relevant literature [Appendix 2]) were linked to EPP frequency, in the multiple species, a multiple regression test was conducted. I also conducted a multiple regression test aiming to detect any association between mating system, and the overall within-song complexity score.

2.2.6 Sexual dichromatism

I also controlled for sexual dichromatism, variation in plumage colour, in all 78 species (as per Garamszegi & Møller 2004, but see Huang & Rabosky 2015) as this has been linked with variation in songbird EPP frequency. Garamszegi & Møller (2004) suggested that of possible confounding factors, only sexual dichromatism had a statistically significant relationship with EPP frequency. There is no evidence that aspects such as habitat or migration had a relationship with EPP frequency. Therefore, these factors were not included in this analysis in terms of potential confounding variables. Sexual dichromatism was categorised on a scale (zero intersexual difference in plumage i.e. monochromatic (scored 0); minimal degree of plumage colour dichromatism (1); moderate dichromatism (3); and male with full colour, and the female brown (5), Simpson et al. 2015) based on species descriptions (Perrins 2009). A multiple regression test was conducted to ascertain whether sexual dichromatism had a relationship with EPP frequency in the multiple study species. I also conducted a multiple regression test to try and detect any association between sexual dichromatism, and the overall song complexity score described above.

2.2.7 Parental care

I controlled for parental care (e.g. rate of nestling provisioning) for all 78 species by testing for correlations between parental care and EPP frequency. Parental care for all species was categorised by: female only (1), male only (2), combination of male and female (3) or communal feeding (4) according to relevant literature (Appendix 2). A multiple regression test was conducted to ascertain whether parental care had a relationship with EPP frequency in the multiple study species. I also conducted a multiple regression test to try and detect any association between parental care and the overall song complexity score. All analyses were conducted using SPSS (version 20.0, IBM, Chicago, IL, USA).

2.2.8 Song variation and data analysis for tui song classes

To compare tui song complexity between BS and IS, one song per class per individual was included in analyses, if indeed both song classes were produced during the recording session. A single song was chosen from each individual and song class at random. For three tui, no IS recordings were obtained. The four within-song complexity variables used for the across-taxa analyses were extracted for this part of the analysis. Two additional within-song complexity variables were also used (trill duration and trill proportion). These two variables are consistent characteristics of tui vocalisations but are not produced by all songbird species which is the reason it was not used for the across-taxa song analysis. This was conducted using Raven Pro (Figure 3a, 3b, Table 2.2) (Hill et al. 2013). Between-song complexity analyses were not conducted in the tui song class analysis because there was an insufficient number of IS available for a comparative analysis with BS.

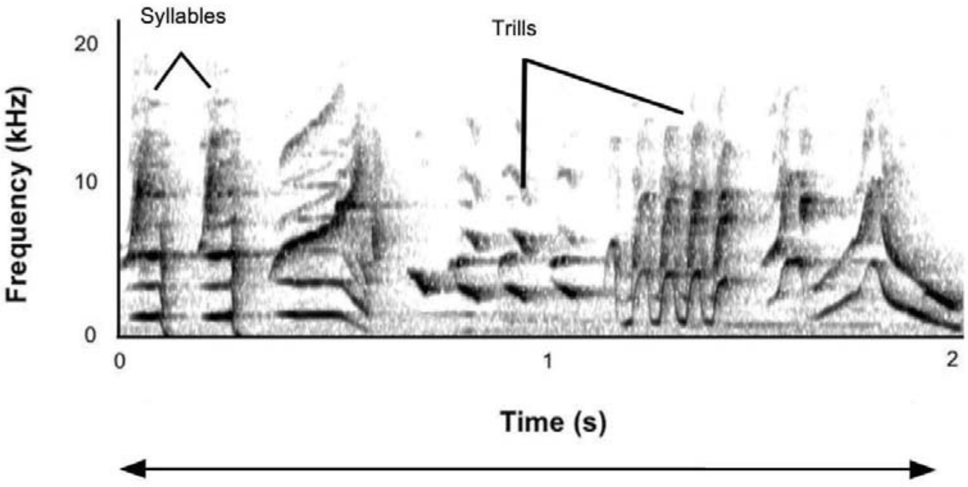
2.2.9 Within-group multivariate analysis

All song complexity data were log-transformed to remove skewness and converted to Z-scores. Z-scores are used to standardise data from different distributions to provide a method of comparing them regardless of their respective distributions (Abdi 2007). Different IS types were produced, male to a male, male to a small group of males and male to a female. A one-way analysis of similarity (ANOSIM, Clarke & Warwick 1994) was used to detect whether song complexity between these different IS types was significantly dissimilar across samples. ANOSIM is a non-parametric multivariate test based on, in this instance, Euclidean distance measure, using the rank order of dissimilarity values to test whether song complexity using all six variables differed between IS types. A one-way permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) based on Euclidean distance measure was also used to test whether within-song complexity using all six variables differed between IS types. These analyses were conducted prior to multivariate comparisons between BS and IS. This allowed us to ascertain whether different IS could be pooled before comparisons with BS.

2.2.10 Between-group multivariate analysis

The variables from both BS and IS were then examined by one-way ANOSIM and PERMANOVA tests to detect whether song complexity between BS and IS was significantly dissimilar. A non-metric multidimensional scaling (NMDS) ordination plot was then generated to provide a visual summary of the patterns of Euclidean values among the samples. All ANOSIM and PERMANOVA tests were conducted in conjunction with the conservative Bonferroni correction procedure for multiple hypothesis testing. The ANOSIM, PERMANOVA and NMDS analyses were all conducted using PAST software (version 2.17b) (Hammer et al. 2001).

(a)



(b)

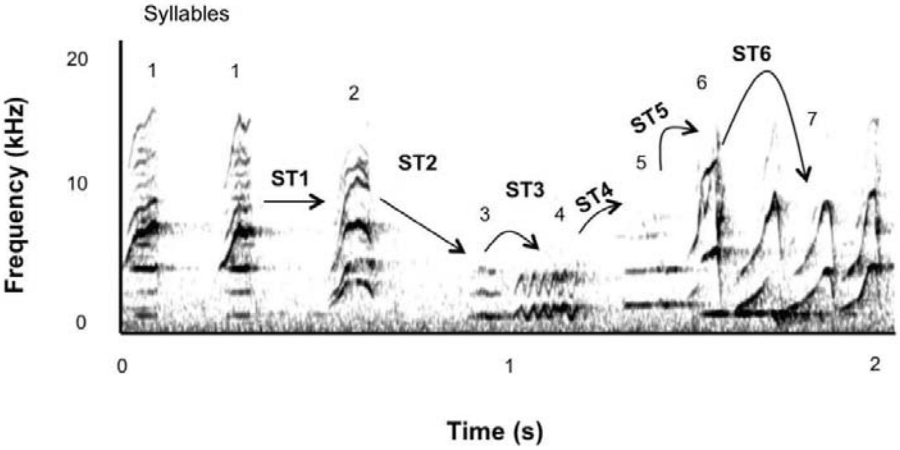


Figure 2.3: Spectrographic representation of the variables employed to measure song complexity in this study. Spectrograms show the a) male tui BS, including trills, which were only used in the analysis of tui song classes as it is a consistent feature of tui songs, and b) a tui BS showing how the number of syllable transitions (ST) were calculated for each song across all species.

2.2.11 Song variables

To compare tui song complexity between BS and IS, one song per class per individual was included in analyses, if indeed both song classes were produced during the recording session. A single song was chosen from each individual and song class at random. For three tui, no IS recordings were obtained. Six within-song complexity variables (including the four used for the across-taxa analyses) were extracted from each tui song using Raven Pro (Figure 2.3a, 2.3b, Table 2.2) (Hill et al. 2013).

2.3 Results

2.3.1 Testing association between EPP and song complexity in multiple species

A linear regression analysis indicated there was a statistically significant positive relationship between EPP frequency, and the overall within-song complexity score in multiple species ($N = 78$; $R^2 = 0.08$; $P = 0.012$) in addition to syllable diversity ($R^2 = 0.1$; $P = 0.003$) and the number of syllable transitions per song ($R^2 = 0.1$; $P = 0.005$), but weaker associations with the number of syllables per song ($R^2 = 0.03$; $P = 0.06$) and song duration ($R^2 = 0.02$; $P = 0.12$). This suggests that EPP frequency predicted overall within-song complexity in the sampled dataset of 78 songbird species (Figure 2.4, Table 2.3).

A linear regression analysis also indicated there was no significant relationship between EPP frequency, and between-song complexity parameters song repertoire size ($N = 76$; $R^2 = 0.06$; $P = 0.52$), syllable repertoire size ($N = 76$; $R^2 = 0.02$; $P = 0.12$) and song versatility ($N = 76$; $R^2 = 0.03$; $P = 0.13$).

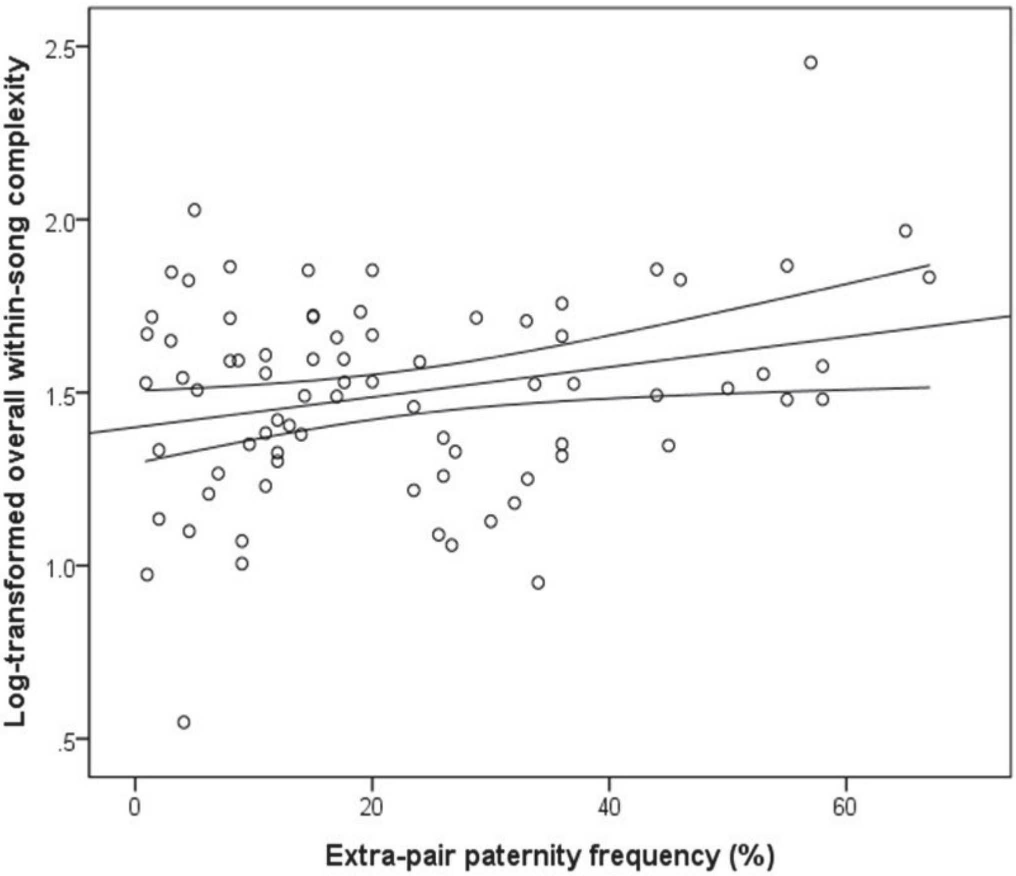


Figure 2.4: The relationship (non-phylogenetic linear regression with mean +/- 95% confidence intervals) between EPP frequency and overall song complexity (log-transformed).

Table 2.3: A summary of the linear regression scores for the relationship between EPP frequency and each of the song complexity parameters.

	EPP frequency		
	R^2	T	P
Number of syllables per song	0.03	1.89	0.06
Overall within-song complexity	0.08	2.58	0.012
Song duration	0.02	1.59	0.12
Song repertoire size	0.06	0.65	0.52
Song versatility	0.03	-1.55	0.13
Syllable diversity	0.1	4.99	0.003
Syllable repertoire size	0.02	1.56	0.12
Syllable transitions	0.1	4.54	0.005

2.3.2 Phylogenetic correction

We conducted a PGLS analysis to correct for phylogenetic relatedness for EPP frequency and song complexity variables. The PGLS estimated λ to be not significantly different from zero for the song complexity traits that exhibited a significant association with EPP in the linear regression analysis (Table 2.4). This suggests that the traits and the observed association between traits were independent of phylogeny and showed greater divergence between taxa than would be expected under a Brownian model of trait evolution.

Table 2.4: The relationship between EPP frequency and the song complexity variables that exhibited a significant association with EPP frequency in a linear regression analysis, tested using PGLS analysis. The *P*-values here signify that the Pagel's λ values for these variables were not significantly different from zero. This suggests that these traits showed greater divergence between taxa than would be expected under a Brownian motion model of trait evolution (i.e. these traits evolved independent of phylogeny).

	EPP frequency		
Song variable	Lambda λ	AIC	*P*
Number of syllables	0.47	621.16	0.34
Overall song complexity	0.61	766.33	0.24
Song duration	0.6	545.64	0.2
Syllable diversity	0.73	572.8	0.17
Syllable transitions	0.34	468.8	0.32

2.3.3 Mating system, sexual dichromatism and parental care

A multiple regression test indicated there was no significant association between EPP frequency and mating system ($N = 78$; $R^2 = -0.03$; $P = 0.065$), sexual dichromatism ($N = 78$; $R^2 = -0.03$; $P = 0.553$) and parental care ($N = 78$; $R^2 = -0.03$; $P = 0.157$).

A multiple regression test also indicated there was no significant association between the overall song complexity score and mating system ($N = 78$; $R^2 = 0.01$; $P = 0.338$), sexual dichromatism ($N = 78$; $R^2 = 0.01$; $P = 0.455$) and parental care ($N = 78$; $R^2 = 0.01$; $P = 0.218$). These results suggested neither mating system, sexual dichromatism or parental care were confounding variables for this study.

2.3.4 Tui song classes: within-group (IS) comparison

Comparison between male-male IS ($N = 27$) and male-female IS ($N = 18$) yielded no significant differences for all six variables (ANOSIM: $R = -0.042$; $P = 0.729$; PERMANOVA: *Pseudo* $F_{38,\ 45} = 2.353$; $P = 0.092$). All IS data were subsequently pooled for comparison with BS.

2.3.5 Comparison between tui BS and IS

There was a significant overall difference between BS and IS of male tui using all six variables measuring within-song complexity (ANOSIM; $N = 48, 45$; $R = 0.409$; $P < 0.001$ with Bonferroni Correction, 9999 permutations, PERMANOVA, $N = 48, 45$;

Pseudo $F_{44,51} = 27.38$; $P < 0.001$ with Bonferroni Correction, 9999 permutations) (Figure 2.5, Table 2.5). Broadcast songs had higher song complexity than IS.

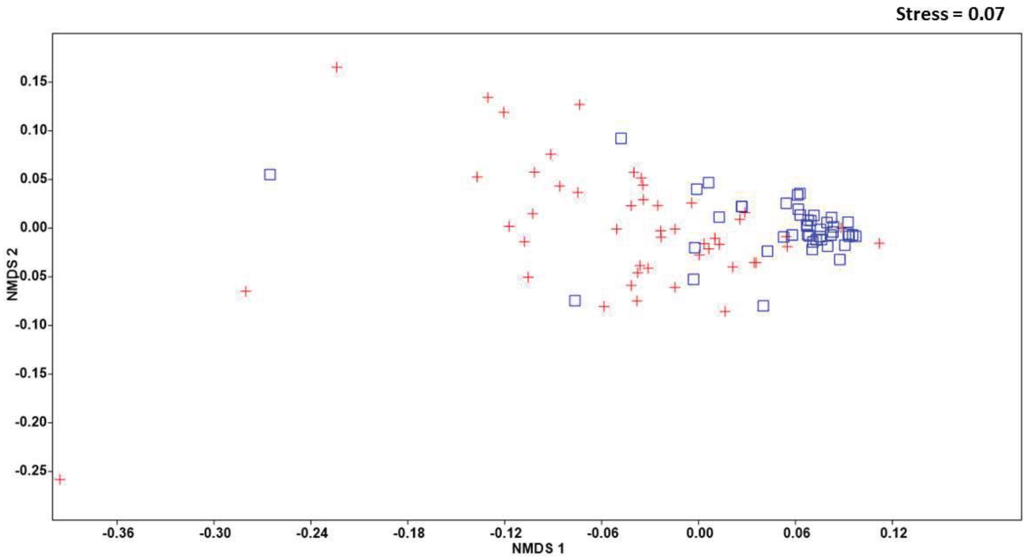


Figure 2.5: The NMDS ordination plot based on Euclidean distance of BS (red crosses) and IS (blue squares) in tui.

Table 2.5: Differences in song complexity parameters between BS and IS of male tui (*N* = 48, 45).

Variable	Mean ± SE	
	Broadcast song	Interactive song
Number of syllables	37 ± 3	21 ± 2
Song duration (seconds)	10.09 ± 1.15	5.2 ± 0.49
Syllable diversity	23 ± 2	12 ± 1
Syllable transitions	21 ± 2	10 ± 1
Trill duration (seconds)	4.59 ± 0.86	1.23 ± 0.36
Trill percentage (%)	44 ± 3	16 ± 3

2.4 Discussion

I assessed the EPP frequency and song complexity associations in a wide range of songbird taxa with varying degrees of EPP. The results indicated a significant positive association in multiple taxa between EPP frequency and overall within-song complexity but not with any between-song complexity variables. Species with higher EPP frequency produced more complex songs overall. In other words, these findings suggest complex vocal traits were ostensibly more pronounced in songbird species with higher EPP frequency. Although more work is still needed to resolve the biological relevance of this statistically significant relationship, the results suggest that EP mate selection may play a role in within-song complexity evolution. Further research is also needed in the vocalisations of females of these species. This would help to ascertain whether female BS complexity also has a relationship with EPP frequency.

Previous studies have suggested that aspects of song such as large individual song repertoires, song rate, song consistency, in addition to the ability to produce and maintain high sound amplitudes, are positively associated with EPP frequency (Hasselquist et al. 1996; Forstmeier 2002; Suter et al. 2009; Taff et al. 2012). Higher song complexity in BS may have therefore partly evolved to maximise the chances of obtaining both social mates and multiple EP mates. However, a previous review study employing a meta-analysis found no relationship between songbird song complexity and EPP frequency (Garamszegi & Møller 2004). These conclusions were supported to some degree by Soma & Garamszegi (2011) who stated multiple biological factors needed to be considered to fully understand the role song plays in reproductive success, such as differences in breeding systems between species. This study revealed significant associations between EPP frequency and a specific key aspect of song complexity (within-song complexity). Within-song complexity is perhaps more useful in measuring the organisation of song components (Sasahara et al. 2012) than a mere calculation of repertoire size or versatility. For example, some species such as chaffinches (*Fringilla coelebs*) sing two to three basic song types (i.e. a small song repertoire). However, within these song types, differences in complexity can be substantial (i.e. high song complexity) despite each song having the same basic pattern (Leitão et al. 2006). Furthermore, higher within-song complexity can evoke stronger responses in both males and females suggesting it evolved under both inter- and intra-sexual selection pressures (Leitão et al. 2006).

Within this study, there was no evidence of a significant association between EPP frequency and between-song complexity. One constraint of this aspect of the study however was the limited number of songs available and analysed per individual although an average was obtained for each species for each variable. Nevertheless, previous research in concordance with this study found neither ubiquitous nor compelling evidence of female sexual selection for larger song repertoires; rather, they suggested the relationship between song and reproductive success evolved as a by-product of social conditions favouring the evolution of complex signals (Byers & Kroodsma 2009). Other studies found that female sedge warblers (*Acrocephalus schoenobaenus*) select EP males with smaller repertoires than their social partners (Marshall et al. 2007). Conversely, some studies have indicated that large song repertoires are associated with greater reproductive success and therefore help attain direct fitness benefits. For example, male song sparrows (*Melospiza melodia*) and willow warblers (*Phylloscopus trochilus*) with large song repertoires had greater reproductive success than those with smaller repertoires (Catchpole 1986; Gil & Slater 2000), although larger repertoires have been positively associated with breeding experience in some species (Motes-Rodrigo et al. 2017). Evidence also suggests in species with polygynous mating systems, males with large song repertoires sire a larger number of offspring with greater viability (Hiebert et al. 1989; Hasselquist 1998). Females mating with males that have large repertoires can therefore gain both direct fitness and indirect genetic benefits for their young (Westneat & Fox 2010). Nonetheless, inconsistencies in conclusions concerning associations between reproductive success and song complexity highlight the need for further clarity and scrutinisation in this area.

From an evolutionary perspective, my study contributes to some extent to the understanding of the life history of songbird species. Extra-pair paternity frequency however can also exhibit within-species variation. This may be caused by differences in stochastic local ecological and genetic factors (Arnold & Owens 2002; Kokko & Mappes 2013). These include differences in aggression of male territory holders (Moulton et al. 2013) and variations in genetic diversity within different populations (Liu et al. 2015). Within-species EPP frequency variation should therefore also be the focus of future field studies in association with song complexity across-taxa as this is still not well understood. The accessibility and advancement of techniques in gathering genetic paternity data in songbirds should facilitate such research. These will help evaluate further the associations of song complexity and EPP.

In tui, a species with high EPP frequency, BS were more complex than IS, consistent with the prediction. This was reflected in a higher number of syllables per song and syllable diversity, in addition to longer song and trill duration and a higher trill percentage in BS. Higher BS complexity in high EPP frequency species could function to maximise both the chances of obtaining social mates, and multiple EP mates. This will need further testing however as BS in addition are likely to have multiple biological functions such as social mate attraction and territory tenure and defence because high song complexity is a signal assessed by both males and females (Leitão et al. 2006). Because BS are designed for long distance signalling, they may also function to minimise social partner cuckoldry.

Higher complexity in tui BS contrasts with earlier studies in the dark-eyed junco (Titus 1998), in which complexity was found to be higher in IS than in BS. The dark-eyed junco has a lower EPP frequency (~27%) than tui (57%). Similarly, in species with lower EPP frequency such as song sparrows (~24%, Hill et al. 2011), male-male interactive aggressive ‘warbled, soft’ songs are not only more complex than BS but are also more variable and exhibit less stereotypy than BS (Anderson et al. 2007, 2008). This suggests increases in BS song complexity are under stronger sexual selection in high EPP frequency species. Further studies however are required to clarify whether the disparities in complexity between BS and IS across all taxa are associated with EPP frequency.

Higher proportions of trills were also found in tui BS compared to IS. Rapid trill components in songbird songs are costly to produce and are thought to be assessed by females as they are indicators of body condition and genetic quality (Ballentine et al. 2004; Hennin et al. 2009). Furthermore, trill consistency is positively associated with male EPP frequency (Cramer 2013) and trills are also thought to be important in male territory defence (Secondi et al. 2002). Tui may produce more complex songs with a greater trill proportion in BS to deter potential rivals (Hill et al. 2015). This would function to minimise the risk of losing paternity via EP copulations (e.g. Reichard et al. 2011).

Extra-pair paternity frequency varies in multiple songbird species and is correlated to the degree of song complexity. My results suggest EP mate selection may play a role in within-song complexity evolution in songbirds. This study contributes to our understanding of variability in songbird within-song complexity between species, a phenomenon that has puzzled ecological and evolutionary researchers. Further research is required to investigate the influence of within-song complexity on the reproductive fitness of individuals in a within- and extra-pair paternity framework to confirm the role of male song complexity in EPP mate selection.

3 Higher song complexity and intruder pressure at dawn in a vocally complex songbird

Male tui displaying the distinctive iridescent plumage and frontal plume feathers (photo: Andrew Underwood, with permission).

This chapter was published in *Emu* (Appendix 1).

Abstract

Dawn is a critical period for vocal displays in songbirds. At dawn, songbirds intensify their vocalisations, for example increasing their singing rates in response to increased intruder pressure at this time, and for mate guarding. However, little is known about diurnal variations in song complexity, a sexually-selected vocal trait associated with mate choice and territorial defence. I compared song complexity in 17 territorial male tui (*Prosthemadera novaeseelandiae*) between dawn, solar noon and dusk. In addition, I investigated whether song complexity correlated directly with rival intrusion rate. The results indicated that song complexity and intrusion rate were significantly higher at dawn than dusk. Analyses also suggested that higher entropy (spectral complexity) and syllable rate, in addition to shorter song duration, were associated with reduced intrusion rate into the singer's territory. Males with higher song complexity may be perceived as stronger competitors and therefore would attract lower rival intrusion. This study presents evidence of both diurnal changes in tui song complexity and associations between song complexity and intrusion rate. These findings support the theory that dawn is important for songbird vocal communication in terms of territory assertion.

Keywords: diurnal variation, songbirds, song complexity

3.1 Introduction

Songbirds use song as a means of communication with two primary functions: mate attraction and territory defence (Andersson 1994; Nordby 1999; Catchpole & Slater 2008; Brumm & Ritschard 2011). Evidence suggests that aspects of song vary across the diurnal period (Avey et al. 2008). To successfully defend a territory throughout the breeding season, singing during the dawn period is particularly important (Kunc et al. 2005). In many songbird species, male vocal activity peaks at dawn, when broadcast songs are produced most intensively (Dabelsteen & Mathevon 2002; Sandoval & Mennill 2014). For example, field sparrows (*Spizella pusilla*) sing just two song types (one complex and one simple) and will produce their complex song type most frequently during the dawn chorus and territorial bouts (Nelson & Croner 1991). Several warbler species may produce songs with unaccented terminal syllables at dawn and dusk compared to other times of day which may aid attenuation of territorial invasions (Morse 1989). Furthermore, song output (number of songs produced) is greater at dawn than at dusk in winter wrens (*Troglodytes troglodytes*, Erne & Amrhein 2008) and rusty-crowned ground-sparrows (*Melozone kieneri*, Sandoval & Mennill 2014). It has been suggested that the intensification of vocal characteristics at dawn may be associated with greater mating activity (Harper 1988; Dabelsteen et al. 1998; Dalziell and Cockburn 2008) and intensified mate guarding (Otter et al. 1997) during dawn. Higher song output for example functions to prevent the singing male from being cuckolded (Møller 1991). However, alternative explanations suggest that production of intensified vocal characteristics were due to calmer weather conditions during the dawn period which is optimal to produce more elaborate vocalisations due to low signal attenuation (Kacelnik & Krebs 1982; Stanley et al. 2016). Male dawn performance is also associated with a male's general ability to defend his territory (Poesel et al. 2004). These findings highlight the importance of vocal intensification during the time of day when intruder pressure or intrusion rate (IR) from rivals is generally at its peak (Catchpole & Slater 2008).

There is conflicting evidence about how energetically and physiologically costly complex songs (i.e. those with long duration, high syllable diversity and high entropy) are to produce. Earlier research (Nottebohm et al. 1981; Otter et al. 1997) indicated complex songs are costly, however more recent research found that song production in some species (e.g. pied flycatchers, *Ficedula hypoleuca*) is not energetically demanding (Ward et al. 2004). The ability to produce complex songs can be impaired by parasites and developmental stress, and reflects the nutritional condition during the early song learning stage (Nowicki et al. 1998; Spencer et al. 2003; 2005). Song complexity can also reflect future reproductive success (Soma & Garamszegi 2011) in addition to cognitive performance (Boogert et al. 2011). Consequently, high song complexity can be considered an honest signal reflecting overall male quality, and is a product of songbird sexual selection (Otter et al. 1997; Buchanan & Catchpole 2000). Female birds will preferentially select males exhibiting these energy-expensive song features (Vallet et al. 1998; Okanoya 2004; Leitão et al. 2006). This is likely to be a factor which evokes strong male responses in terms of physical aggression and intensified song performance.

Song complexity is a vocal behaviour that may be used by males to assess rivals (Mountjoy & Lemon 1991) subsequently stimulating agonistic approaches from males competing for territories. Song complexity also reflects adult body condition, which is critical to successfully defend territories (Lampe & Espmark 1994; Reid et al. 2005; Kipper et al. 2006; Potvin et al. 2015) as it may act as a signal to other males of the guard's ability to defend his territory (Lampe & Espmark 1994; Kipper et al. 2006). A complex song produced by an invader may indicate a stronger challenge to a male's territory. Accordingly, this evokes stronger responses from the territory holder to minimise successful territorial invasions by conspecific males. Males that produce simple songs are more likely to become the victim of territorial invasions (Falls 1988; Mountjoy & Lemon 1991), and, conversely, complex songs should reduce the number of territorial invasions. There is evidence that suggests the presence of large song repertoires can reduce the IR from rival males (Yasukawa 1981). As described above, multiple vocal aspects are intensified or increased at dawn including song output, unaccented terminal syllable production, and production of the more complex song type in a species where only one complex and one simple song type are produced. Therefore, it would be predicted that song complexity would also be increased at dawn, however this remains untested.

Tui (*Prosthemadera novaeseelandiae*) are endemic New Zealand honeyeaters (Order Passeriformes, Suborder Oscine, Family Meliphagidae). They exhibit strong territoriality, sexual size dimorphism and a high rate of extra-pair paternity (57% of sampled offspring sired by extra-pair males, Wells et al. 2015). Males and females both sing, however females sing far less frequently (Hill et al. 2017), and have smaller syllable repertoires than males (Hill 2011). Male broadcast song is the most frequently produced song type, whereas intra- and intersexual interactive songs are far less frequent (Hill et al. 2017), and are produced in territorial, courtship or pair-bond contexts (Hill 2011). Both geographical (Hill et al. 2013), and seasonal variation (Hill et al. 2015) in tui song complexity have been shown. Little is currently known about sexually-selected tui song features, however, in multiple songbird species song complexity is strongly selected by females, as complex songs are potentially costly signals to produce (Nottebohm et al. 1981; Otter et al. 1997), and so we would predict tui song complexity to show similar trends. To further examine intensification of songbird vocal characteristics at dawn, I examined the diurnal variation in tui song complexity, and a possible correlation between song complexity and IR in adult male tui. As vocal intensification can serve to reduce IR in many species (Yasukawa 1981; De Kort et al. 2009), I predicted that dawn tui songs would be intensified (i.e. more complex) than solar noon and dusk songs, functioning at least partly to mitigate increased dawn IR from rival males. Hence, I also predicted that individual tui producing more complex songs would suffer fewer IR. This study therefore contributes to the understanding of tui song complexity variation in the context of male territory defence.

3.2 Methods

3.2.1 Study site

This study was conducted across three locations, Jones Bay, the lagoon area, and the Ecology Bush, at Tawharanui Regional Park in New Zealand (Figure 3.1). The 588 ha park consists of bush remnants separated by pasture where there is an estimated tui population of approximately 2000 individuals (Wells et al. 2015). Jones Bay consists of large open pastureland with patches of native trees (kanuka *Kunzea robusta*, karo *Pittosporum crassifolium*, and pohutukawa *Metrosideros excelsa*), and exotic species such as flame trees (*Erythrina x sykesii*) (Hill et al. 2017). The lagoon area of the park consists predominantly of pastureland with patches of kanuka, and manuka (*Leptospermum scoparium*), with sporadic pohutukawa. The Ecology Bush area of the park consists predominantly of mature coastal forest remnants. Tree species within this area include kanuka, nikau (*Rhopalostylis sapida*), puriri (*Vitex lucens*), rewarewa (*Knightia excelsa*), taraire (*Beilschmiedia tarairi*), and tawa (*Beilschmiedia tawa*) (Hill et al. 2017).

Figure 3.1: Map of Tawharanui Regional Park showing the three study locations where tui were recorded and observed within the park (1. Jones Bay, 2. Lagoon area, 3. Ecology Bush).

3.2.2 Song recording

Song recordings were made across three tui breeding seasons (October-February), from 2012 to 2015 in clear, still and dry climatic conditions. The breeding territories of 17 adult male tui across the three locations within Tawharanui were identified. Territory boundaries were ascertained by watching flights and movements of male birds (Ritchison 1988). Songs of these tui were recorded, 11 individuals in 2012/2013, two in 2013/2014 and four in 2014/2015. Six of these tui were recorded in Jones Bay, four individuals were recorded in the lagoon area of the park, and seven individuals were recorded within their territories within the Ecology Bush area of the park. Exact territory measurements were not made, however from observations, territories varied in size, with the largest territories seemingly being around the lagoon area and the smallest within the Ecology Bush. The visibility for tui and for human observers was generally clearest within the lagoon area, and within the Ecology Bush the least clear due to high plant density. Nevertheless, the focal birds were always able to be visually and/or audibly tracked during the recording sessions. All focal birds were colour banded and individually identifiable. Female partners of five of these males were observed but none of these females were observed singing during male broadcast songs. More males may have had breeding partners, but this could not be confirmed therefore the reproductive status and breeding stage of 12 males was not established. We also cannot confirm whether the females observed with the resident males were within- or extra-pair partners.

Tui generally began singing ~60 minutes before official sunrise time (S. D. Hill personal observation 2014), which was selected as the initiation time for song recording, and was determined by using timeanddate.com (https://www.timeanddate.com/sun/). Three x three-hour recording sessions were conducted on each recording day for 1) dawn: 1 hour before official sunrise time until 2 hours after official sunrise time; 2) solar noon: 1.5 hours before official solar noon until 1.5 hours after official solar noon; and 3) dusk: 2 hours prior to official sunset until 1 hour after official sunset (tui generally ceased singing prior to 60 minutes after official sunset, S. D. Hill personal observation 2014). This temporal recording session structure was adapted from previous studies (Tomialojc & Lontkowski 1989; Senar et al. 1992; Thomas 1999; Avey et al. 2008; Erne & Amrhein 2008). Every song recorded from each bird, with a good signal-to-noise ratio, was used in the analysis to compare song complexity between dawn, solar noon, and dusk. All analysed recordings had similar low levels of background noise (e.g. from climatic factors and vocalisations from other birds). Only one banded individual was recorded on any given day for all three time periods. Each banded bird therefore was recorded for only one day. Some individuals did not produce any songs for some time periods on their respective recording days, yet sampling effort remained consistent. Hence, minor sample size discrepancies existed between times of day. Recordings were made using a Sennheiser ME67 long-range directional microphone (Sennheiser, Old Lyme, CT) with a frequency response range of 40 Hz to 20 kHz, attached to a Marantz PMD620 digital recorder (Marantz, Kanagawa, Japan). All recordings were written as wav files at a sampling rate of 44.1 kHz, with a resolution of 24 bits.

3.2.3 Intrusion rate (intruder pressure)

Intruder pressure was quantified by tallying the number of intruder visits (IR) to an individual's territory (Stamps et al. 1987) over a unit time. I assessed which time of day (dawn, solar noon or dusk) had the highest IR. During each recording session, I counted the number of male-male agonistic encounters observed between each resident focal male and males that intruded their territory within a 10-minute continuous sampling period (see Tacha et al. 1985) providing a total of 30 minutes per day in total for each individual. Continuous observations are recommended for studies where the aim is to generate the frequency of a specific behaviour (Tacha et al. 1985). During dawn and dusk sessions, it was ensured these counts were conducted in good visibility (i.e. after official sunrise and before official sunset).

During solar noon sessions, these counts were conducted before official solar noon. For each individual raw IR data were converted to hourly rates by multiplying by six for the purposes of standardisation for data analysis. The conversion to an hourly rate rescales the data into an interpretable unit and allows comparison with other studies such as in red-winged blackbirds (*Agelaius phoeniceus*, Searcy & Yasukawa 1995). From observations, when a rival male intruded a territory and landed on a perch within the territory, resident males would generally aggressively approach them. Interactive song between the perched individuals generally ensued after the resident male landed on the perch. A male-male encounter was only tallied as an intrusion if an intruding male was chased out of its territory. Interactions ultimately resulted in intruder departure. For intruding males that were not colour banded, I accept there was a chance the same individual may have intruded the focal birds' territory on more than one occasion. Only one banded focal male was monitored on any given day as per the sound recording procedure.

3.2.4 Song variables

Broadcast songs are the most commonly produced songs in tui and are defined as prominent and non-directional vocal signals. Broadcast songs in songbirds, function as advertisements of reproductive and/or territorial status (Wiley & Richards; Titus 1998). In contrast, interactive songs are often initiated and directed towards an observable audience. They are employed in male-male confrontations (Titus 1998), in addition to courtship and pair-bond maintenance (Titus 1998; Reichard et al. 2011). Variation occurs in both tui song structure and complexity between broadcast and interactive songs (S. D. Hill, unpublished data 2016) and between sexes (Hill 2011). To ensure both a large sample of songs and to avoid the potential confounding effects of song type and sex variation, only male broadcast songs were included in the analysis. All songs included in the analysis were recorded at between 2 m and 5 m from each singing bird.

The song recordings were visualised, and parameters measured using Raven Pro 1.4 Beta Version software (Cornell Lab of Ornithology, Ithaca, NY, USA). For all recordings, spectrograms were created by Discrete Fourier Transform (DFT) with a Hann window. The frame length was set at 256 points. Additionally, a 50% frame overlap with hop size of 2.9 ms was used. Frequency grid spacing of 172 Hz was also employed and the bandwidth was set at 3 dB.

To measure song complexity, I used Raven Pro to extract five variables (Fig. 2):

1. Entropy (a unitless measure of spectral complexity). White noise, for example, has a high entropy score whereas a pure tone has a low entropy score (Tchernichovski et al. 2000; Briefer et al. 2010; Charif et al. 2010). Entropy was measured across the entire song and was calculated by Raven Pro,

2. Number of syllables (sound units per song, Boogert et al. 2008). Within a song, I considered it a different syllable if it was separated from other syllables by a visible temporal interval ≥ 90 ms,

3. Song duration (time from the beginning of the first syllable to the end of the terminal syllable of each chosen song, Boogert et al. 2008),

4. Syllable diversity (number of different syllables per song (Baker & Logue 2003; Garamszegi & Møller 2004; Weir & Wheatcroft 2011). Different syllables were identified and distinguished by duration, spectral structure, and bandwidth (i.e. the difference between minimum and maximum frequencies),

5. Syllable rate (the number of syllables produced per second (Feßl & Hoi 2000). All song complexity parameters have been employed in previous studies (Gil & Slater 2000).

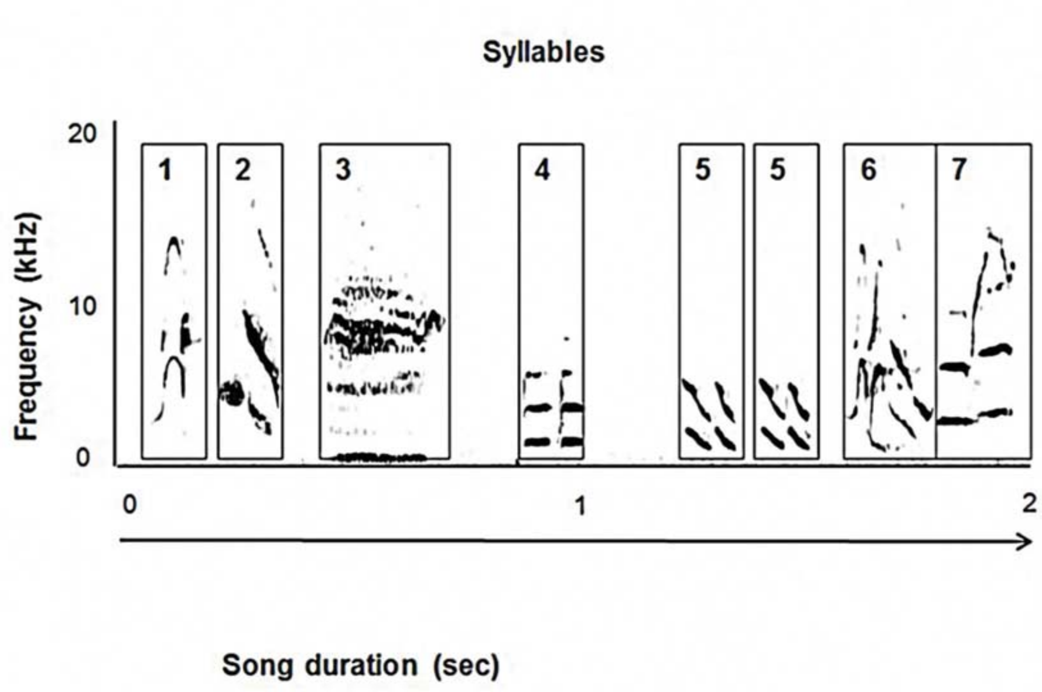


Figure 3.2: A spectrogram of a male tui song highlighting total number of syllables, song duration and syllable diversity. This example has eight syllables in total and seven different syllables (syllable five is repeated). The syllable rate of this song is 8 ÷ 2 sec = 4 syllables per second.

3.2.5 Statistical and graphical methods

A permutational multivariate analysis of variance (PERMANOVA, Anderson 2001, using 999 permutations) was used to examine differences in song variable centroids between three times of day (dawn, solar noon and dusk). The number of songs for each bird varied, so each 'individual bird' was considered a random factor and nested within location. Data were standardised to Z-scores prior to the PERMANOVA. The song complexity data was visualised using non-metric dimensional scaling (NMDS), using the average for each bird at each time of day. I also tested for significant differences in IR between times of day using a one-way ANOVA and *Post hoc* analyses (Tukey's pairwise comparisons).

To examine the relationship between song complexity and IR (per h) we initially used an exploratory factor analysis (EFA) to reduce the number of correlated song complexity variables into principal components (PCs). The function of EFA is to uncover underlying structure and relationships between a multitude of variables. A VARIMAX rotation of the data was performed to try and maximise interpretability of the vectors (i.e. PC1 etc.). The 'shared variances' (of the variables in each PC) were used rather than the 'total variance' of each variable. Each PC therefore represented an orthogonal (i.e. uncorrelated) linear combination of these song complexity variables. These PCs were fit as predictor variables in a model predicting IR. The variation in IR was over-dispersed: the dispersion parameter for the data was 7.4 suggesting the data were more aggregated than would be expected with a Poisson distribution, so a generalised linear model (GLM) with a quasi-Poisson error structure was fit to the data.

The PERMANOVA and NMDS analyses of song complexity data were conducted using the PRIMER v6 computer program (Clarke and Gorley, 2006 with the PERMANOVA+ add-on package (Anderson and Gorley 2008) using Euclidean distance measures. The ANOVA was done using PAST software (version 2.17b, Hammer et al. 2001). The EFA and GLM were fitted using the R package for statistical computing (R Core Development Team 2015).

3.3 Results

In total, 926 songs were analysed from 17 male tui: 401 at dawn (mean per individual ± standard error: 23 ± 6; N = 15), 209 at solar noon (12 ± 4; N = 11) and 316 at dusk (19 ± 3; N = 16). Dawn had generally higher song complexity values than both solar noon and dusk (PERMANOVA test *Pseudo* $F_{11, 17} = 1.862$; $P = 0.049$; Table 3.1; Table 3.2; Table 3.3). Dawn song complexity was positively associated with high number of syllables, song duration and syllable diversity, with datapoints generally distributed towards the right of the NMDS plot. The NMDS also suggests dusk songs had a relatively low number of syllables, song duration and syllable diversity compared to dawn as the datapoints were distributed more towards the left of the plot (Figure 3.3).

Table 3.1: The descriptive statistics showing the average song complexity parameters and intrusion rate at dawn, solar noon, and dusk from 401 broadcast songs at dawn, 203 songs at solar noon, and 316 songs at dusk in a tui population (N = 15, 11, 16).

Variable	Mean ± SE		
	Dawn	Solar noon	Dusk
Entropy (u)	3.69 ± 0.21	3.35 ± 0.25	3.14 ± 0.23
Number of syllables per song	22.28 ± 1.62	17.89 ± 1.56	17.57 ± 1.53
Song duration (s)	8.81 ± 1.11	7.77 ± 1.17	6.27 ± 0.25
Syllable diversity	18.79 ± 1.29	15.64 ± 1.51	15.05 ± 1.26
Syllable rate (per s)	2.13 ± 0.02	2.16 ± 0.2	2.39 ± 0.12
Intrusion rate (per h)	18 ± 3.61	6.35 ± 2.02	5.22 ± 1.64

Table 3.2: A matrix showing the test statistic/*P* values of pairwise PERMANOVA tests that compared song complexity levels between the 3 times of day (999 permutations).

	Dawn	Solar noon	Dusk
Dawn	-		
Solar noon	*Pseudo* F = 1.44 *P* = 0.154	-	
Dusk	*Pseudo* F = 1.862 *P* = 0.049	*Pseudo* F = 1.07 *P* = 0.353	-

Table 3.3: The number of males that either decreased (↓), increased (↑), or remained unchanged (→) for each song complexity variable and IR at dusk compared to dawn. Note that three individuals did not sing during both dawn *and* dusk recording sessions so were omitted from the tally.

Entropy	Number of syllables per song	Song duration	Syllable diversity	Syllable rate	Intrusion rate
6 ↓	8 ↓	8 ↓	9 ↓	6 ↓	11 ↓
8 ↑	6 ↑	6 ↑	5 ↑	8 ↑	1 ↑
					2 →

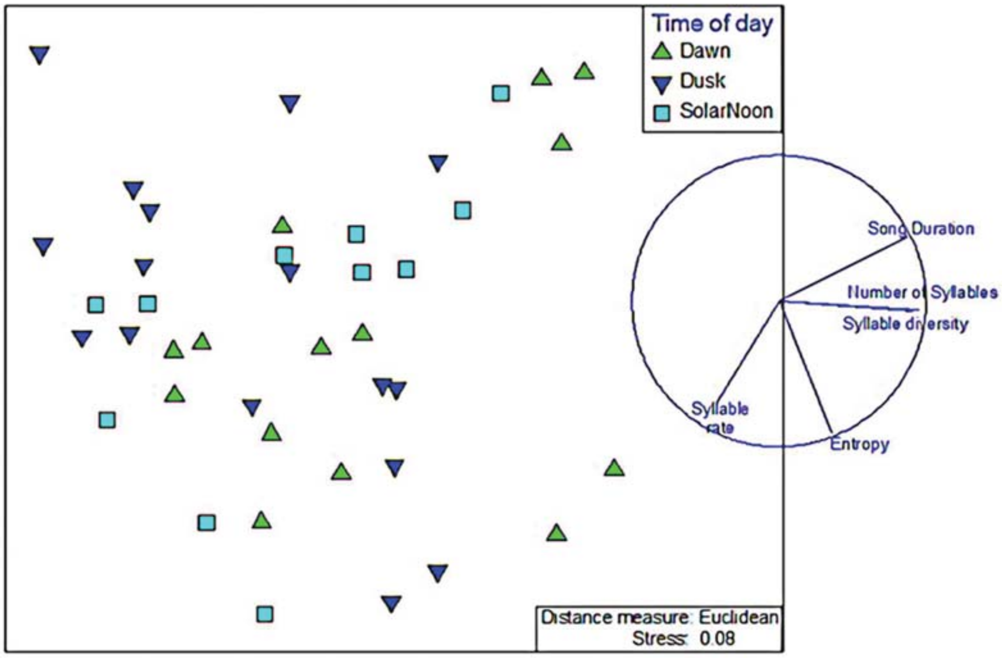


Figure 3.3: NMDS plot of the tui song complexity data showing differences in the centroids of the three different times of day (dawn, solar noon, and dusk). The plot suggests dawn complexity parameters correlate with higher number of syllables, song duration and syllable diversity as the dawn datapoints were situated generally towards the right of the plot. Dusk on the other hand had lower complexity in terms of these variables as the dusk datapoints were situated more towards the left of the plot. The inset shows a unit circle (radius = 1) with vector length describing the Spearman rank correlation of the song variables.

We found evidence of significant differences in IR group means between times of day in all individuals, as determined by a one-way ANOVA test ($F_{2, 48} = 8.596$; $P < 0.001$; N = 17). *Post hoc* Tukey's pairwise comparisons suggested there was strong evidence that dawn IR was higher than at solar noon ($P = 0.004$) and dusk ($P = 0.001$) (Table 3.2).

3.3.1 Relationship between song complexity and intrusion rate

Covariates that were not known to be part of the relationship between song complexity and IR and did not have any evidence of predictive power (i.e. those that did not deviate significantly from zero) were excluded from the model. There was no evidence of any relationship between the first principal component (PC1) of the song complexity data with IR therefore, PC1 was not included in the model. However, PC2 and PC3 were used as covariates in the GLM used to predict IR. Within the EFA, the PC2 axis was highly loaded with entropy while PC3 was heavily loaded with song duration and syllable rate (Table 3.4). There was a trend that IR was negatively associated with PC2 ($R^2 = 0.4$; $P = 0.058$) and IR was negatively associated with PC3 ($R^2 = 0.4$; $P = 0.028$). We estimated that a unit increase in PC2 decreased IR by 29.6% (95%CI [-48.1%, -2.2%]) and that a unit increase in PC3 decreased IR by 17.4% (95%CI [-33.5%, +2.7%]). These suggest reduced tui IR was associated with increased entropy and increased syllable rate/decreased song duration.

Table 3.4: The eigenvalues, variance explained and factor loadings of the variables following exploratory factor analysis for the song complexity variables that were to be used to test for associations with intrusion rate. Those variables with factor loading values greater than 0.8 are shown in bold.

	PC1	PC2	PC3
Eigenvalue	2.88	1.59	0.46
% of variance explained	50	29	21
Entropy	0.19	**0.96**	0.2
Number of syllables per song	**0.96**	0.22	-0.17
Song duration	0.73	-0.08	-0.66
Syllable diversity	**0.98**	0.15	-0.06
Syllable rate	-0.1	0.2	**0.97**

3.4 Discussion

Consistent with the predictions, the results indicated significantly higher tui song complexity at dawn than at dusk, significantly greater IR at dawn than at solar noon and dusk, and an association between IR and three song complexity variables: entropy, syllable rate and song duration. Greater song complexity observed at dawn is concordant with previous research suggesting vocal activity varies throughout the day (Erne & Amrhein 2008). Although currently untested in tui, there are likely to be multiple biological and environmental factors that play a role in song complexity changes over the diurnal period and the high song complexity during the dawn period (dawn chorus). These include microclimatic fluctuations (Kacelnik & Krebs 1982), because favourable microclimatic conditions make dawn an optimal time of day to successfully transmit vocalisations (Kacelnik & Krebs 1982). It has been hypothesised that the diurnal variation in climatic parameters, such as relative humidity, wind speed and temperature, may at least partly explain the evolution of the dawn chorus. Local air turbulence, for example, can cause attenuation of sound and create background noise, reducing the effective transmission of sound and is generally at its lowest at dawn (Kacelnik & Krebs 1982). The production of more elaborate and complex vocalisations would therefore be most ideally suited to dawn as this will maximise sound propagation and decodability (Brown & Handford 2003; Stanley et al. 2016).

However, dawn conditions are not always optimal for long-range communication, therefore the dawn chorus cannot solely be explained by this sound transmission hypothesis (Dabelsteen & Mathevon 2002). Diurnal variation in reproductive behaviour such as both within- and extra-pair copulations (Harper 1988; Dabelsteen et al. 1998) may also explain song complexity variations. However, quantifying these behaviours in wild birds proves challenging, because copulations can often be undertaken out of the researcher's sight. Neurological changes such as the plasticity of songbird brain morphology during the diurnal period (De Groof 2009) may also govern song complexity variation. This has been observed in conjunction with seasonal changes in vocal behaviour (De Groof 2009), which could be influenced by melatonin concentrations which in turn influences song patterns (Derégnaucourt et al. 2012).

There is evidence that the male dawn chorus has a dual function of both enforcement of dominance among male group members in addition to signalling attractiveness to females. Male superb fairy-wrens (*Malurus cyaneus*) for example will seek extra-pair copulations exclusively during the dawn chorus (Dalziell & Cockburn 2008). Furthermore, the dawn chorus in great tits (*Parus major*) is directly related to female fertility (Mace 1987). During the dawn chorus, males increase both their song rate and song duration in the lead up to egg laying, suggesting there are differential functions of song at specific times of day (Dalziell & Cockburn 2008). This suggests the dawn chorus could function as a mate-guarding tactic (Otter et al. 1997), although other evidence suggests that dawn song functions for males to attract their own mates, replacement females for widowed males, and extra-pair mates (Slagsvold *et* al. 1994). Conceivably, song complexity is increased at dawn to deter rivals from entering territories when female partners are fertile and vulnerable to extra-pair copulations (Møller 1988). It may also be due to mated males themselves seeking extra-pair copulations. These factors are however currently undetermined in tui and warrant future efforts.

In addition to tui song complexity, IR was also significantly higher at dawn than at both solar noon and dusk. Tui have high extra-pair paternity rates (Wells et al. 2015) which suggests successful territorial invasions are relatively commonplace in tui. It also means that mate guarding, and territory defence are likely to be of high importance for resident males. Additionally, females may leave territories and seek out extra-pair copulations, but this needs future clarification. My results suggest entropy had a significantly negative association with IR therefore songs with high spectral complexity may be used to some extent to minimise or mitigate these territorial invasions by rivals. Previous evidence indicates that song is used during the dawn chorus firstly for territorial holders to announce territory occupancy to non-territorial males prospecting for territories, thereby reducing the chances of territory seizure, and secondly for non-territorial males to assess territory-holding male quality (Amrhein et al. 2004). Dawn intrusions by prospecting males may also function to assess the availability of a female (Amrhein et al. 2004).

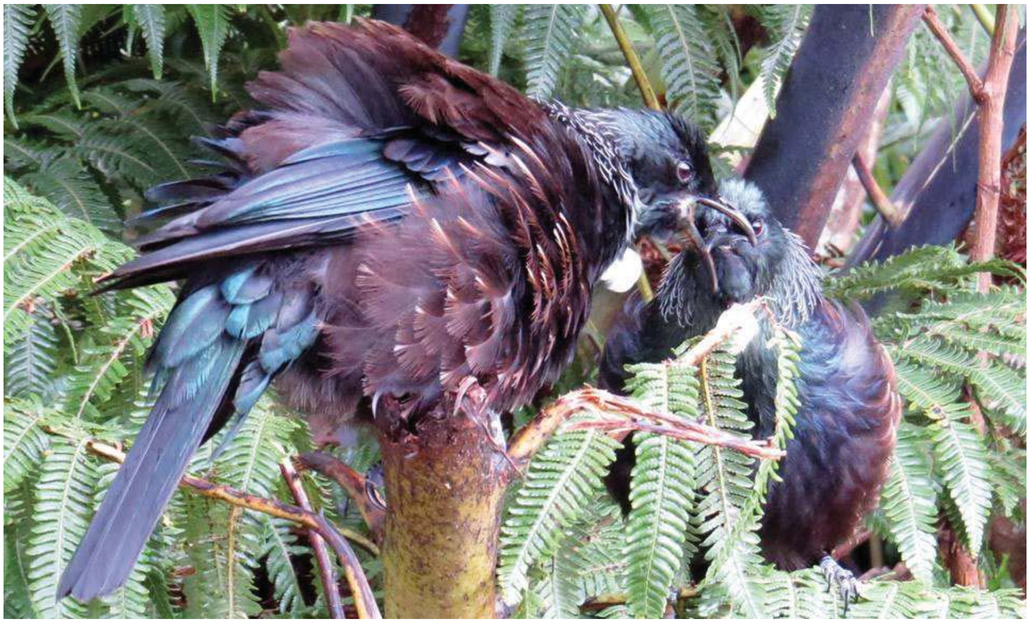
To a lesser extent, songs with increased syllable rate (and decreased duration) were also associated with reduced IR. Previous evidence suggests that in banded wrens (*Thryophilus pleurostictus*), long, high performance songs reduce rival male intrusions (De Kort et al. 2009). This previous study measured the aggression of response to low, medium and high-performance singers. The few individuals that did respond to high performance songs were more aggressive to high-performance songs than to low performance songs (De Kort et al. 2009). I am somewhat cautious of drawing strong conclusions on the association between tui song complexity and intruder pressure because of the low relationship strength found within this study. Furthermore, variations in other territorial variables of the territory holder (e.g. habitat complexity and density, food availability, territory size, reproductive success, age and dominance status) also are likely to influence tui IR, and will need further testing. It should also be acknowledged that increased song complexity may be due to increased IR rather than vice versa, although this hypothesis remains to be tested in tui, and therefore warrants future efforts. These include manipulation experiments with the introduction of varied numbers of dummy male tui models into respective territories and measuring song complexity responses from territory-holding males. With the increasing power and advancement of computer technology, computer-controlled robotic or drone bird models which mimic the exact movements of birds (Zhang & Hu 2016) could be employed to enter territories and simulate intrusion events. The feasibility of using robotic birds in the field of experimental biology has been demonstrated by, for example, Butler et al. (2014) in a study on European starlings (*Sturnus vulgaris*).

Previous research suggests intruder pressure is most ostensive, in some species, at dawn (Dabelsteen et al. 1998). More attempts at agonistic territorial intrusions by rival males at dawn have been detected in previous research (Liu 2004). This suggests the necessity for a greater degree of territory defence at dawn. Males of many songbird species have a peak in their singing activity at dawn (Nelson & Croner 1991). Evidence suggests that singing during these diurnal periods may have several different functions. Many species for example produce shorter and less intense dawn choruses as the breeding season progresses (Poesel et al. 2006) indicating a role in mate attraction. Dawn chorus performance is also positively correlated with the ability of male blue tits (*Parus caeruleus*) to defend territories later in the day (Poesel et al. 2004).

Male songbirds will adjust dawn song output to the level of intruder pressure (Liu 2004; Erne & Amrhein 2008). In my study, the greater number of territorial encounters observed during the dawn session than during dusk could be a key contributory factor to the greater song complexity produced during this period. This is likely to function to guard territory and food resources. In order to test causality between song complexity and IR however I recommend experimental manipulation using song playbacks (with complexity variables set at different values). These experiments should also include the use of male dummy models on territories in conjunction with playbacks and measuring respective IR on different territories.

This study has provided novel evidence for diurnal song complexity variations in tui and therefore provides us with a greater understanding of songbird vocal behaviour alterations across the diurnal period. This research supports the theory that dawn is a critical time of day for the intensification of vocal characteristics in songbirds. At dawn, I found higher entropy, higher number of syllables, higher syllable diversity and longer song duration than at other periods of the day. I also recorded higher tui IR at dawn. Higher spectral complexity, and syllable rate, in addition to shorter song duration, were associated with reduced IR into the signaller's territory. This research thus contributes to the understanding of songbird song complexity function and evolution in relation to territorial assertion, and provides important baseline data that provides a starting point for future efforts in the realms of diurnal changes in songbird communication.

4 Fighting talk: complex song elicits more aggressive responses in a vocally complex songbird



Interactive male-male tui behaviour (photo: Bryan Pepperell, with permission).

This chapter was accepted for publication in *Ibis* (Appendix 1).

Abstract

Song complexity in many songbirds is a trait subject to sexual selection. It is often associated with male territorial defence. Empirical studies testing differential male responses to rival song in vocally complex songbirds however have been scarce. I conducted playback experiments in tui *Prosthemadera novaeseelandiae* to test the response aggressiveness of territorial males to rival songs with differing complexity levels. Overall, complex songs evoked significantly stronger responses from territorial males than simple songs. Following playback of complex songs, focal males approached the playback more closely and rapidly, and responded with songs of higher complexity than to playback of simple songs. This suggests males could both distinguish between different levels of complexity within the tui repertoire, and perceived more complex song as a greater territorial threat. This study is one of the first to demonstrate strong aggressive responses to increased levels of song complexity in a songbird species with highly complex vocalisations. In the context of male territory defence, this study provides important insights into the evolution of complex tui song.

Keywords: playback, rival, simple, song complexity, territoriality

4.1 Introduction

Bird song is a communication signal with two key functions: mate attraction, and territory defence (Catchpole & Slater 2008; Brunner & Pasinelli 2010; Leedale et al. 2015; Moiron et al. 2015; Monbureau et al. 2015). Song production may provide advantages to the singer on both fronts, or the 'dual function hypothesis' (Catchpole & Slater 2008). This becomes particularly important during the female fertile period (Greig-Smith 1982) when competition for breeding partners and resources is at its peak (Hjernquist et al. 2008; Pryke 2013).

Evidence suggests complex songs evolved due at least partly to sexual selection for such high-performance vocalisations (Catchpole 1980). Nonetheless, levels of song complexity (e.g. the number of syllables contained within a song) vary greatly across songbird taxa. This is partly due to intraspecific variations in song structure (DeVoogd et al. 1993). The California thrasher *Toxostoma redivivum* and winter wren *Troglodytes hiemalis* for example produce complex songs containing multiple different syllables (Kroodsma 1981; Cody et al. 2016). On the other hand, the white-crowned sparrow *Zonotrichia leucophrys*, zebra finch *Taeniopygia guttata* and chiffchaff *Phylloscopus collybita* have merely a few similar syllables within their repertoire (Marler & Tamura 1962; Zann 1996; Jaška et al. 2015). Evidence suggests high songbird song complexity is both energetically costly in terms of field metabolic rate (Garamszegi et al. 2006) and sexually selected (Schmidt et al. 2013). Song complexity may be an honest indicator for various aspects of male quality (Spencer et al. 2003; Cramer 2013). These factors include genetic diversity

(Marshall et al. 2003), developmental stress levels (Spencer et al. 2003; Nowicki & Searcy 2004), and parasite load (Hamilton & Zuk 1982).

Song structure may be shaped by the forces of both intra- and intersexual selection (Catchpole & Slater 2008). Levels of song complexity can even vary between individuals of the same species (Feßl & Hoi 2000). Song complexity variation is largely governed by the volume of the motor song system nucleus (HVC, DeVoogd 2004). Song complexity may be used by males to assess the quality of potential territorial rivals in vocally complex species (Mountjoy & Lemon 1991). Moreover, evidence suggests song complexity plays an important role in territory acquisition and defence (Beecher & Brenowitz 2005). Furthermore, males of some species, such as red-winged blackbirds (*Agelaius phoeniceus*), deemphasise their song repertoires in the presence of rival males (West et al. 1981; Searcy & Yasukawa 1990) possibly functioning to avoid costly physical conflict.

Song complexity has also been found to correlate with several reproductive success metrics. For instance, increased parental care (e.g. in sedge warblers *Acrocephalus schoenobaenus*, Buchanan and Catchpole 2000), initial mating success (e.g. song sparrows *Melospiza melodia*, Reid et al. 2004), and lifetime reproductive success through territory tenure (e.g. song sparrows, Potvin et al. 2015). Furthermore, males with high song complexity pair earlier (e.g. European starlings *Sturnus vulgaris*, Mountjoy & Lemon 1996), and are more successful in extra-pair breeding (e.g. great reed warblers *Acrocephalus arundinaceus*, Hasselquist et al. 1996; Leisler et al. 2000).

Playback experiments under natural conditions can be highly powerful tools to investigate bird song variation, elucidate the rudimentary functions of song and examine the roles of aspects of song structure (Catchpole et al. 1984; Kroodsma & Byers 1991; Kipper et al. 2015). Playbacks combine both the precision and accuracy of laboratory conditions with the unknown, uncontrollable, natural conditions of the field (Falls 1992). However, birds that have been bred in laboratories are often domesticated and may exhibit different traits than their wild congeners (Campbell et al. 2009). For example, captive birds can have poorer body condition and immune systems than wild individuals (Ewenson et al. 2001). Field studies therefore have a major advantage because they are conducted within a natural context, in which animal behaviours are most uninhibited (Falls 1992). Their responses are therefore likely to reflect biologically relevant behaviours (Falls 1992). Such playback experiments can subsequently provide valuable insights into evolutionarily important facets of animal communication (Fischer et al. 2013). Playback experiments investigating female responses to male songs for example found that song complexity plays a part in female mate choice (Howard 1974; Catchpole 1980; Searcy 1992; Catchpole & Leisler 1996).

The tui *Prosthemadera novaeseelandiae* is an endemic New Zealand songbird in the Family Meliphagidae. Both sexes sing, however females less frequently (Bergquist 1989). Tui produce long-range, territorial broadcast songs generally long and complex in nature (Hill et al. 2015). They will also produce shorter and simpler broadcast songs in addition to alarm and distress calls. These two types of broadcast song may have different functions but this is unclear. Males can produce at least 56 different syllables within a single song (Hill et al. 2015). This large syllable repertoire results in a large and diverse song repertoire. Indeed, male tui songs are rarely repeated as exact copies (Hill 2011) and within-individual song complexity varies (Hill et al. 2015). Some syllables and song strophes are nevertheless shared between neighbouring individuals (Hill & Ji 2013). While the functions of tui song remain largely unelucidated, evidence suggests they may be used in mate attraction, since songs are more complex during breeding season (Hill et al. 2015), dominance hierarchy resolution (Bergquist & Craig 1988; Bergquist 1989) and territoriality (Hill et al. 2013). Tui also have high extra-pair paternity (57%, Wells et al. 2015). This suite of behavioural traits makes tui an excellent model to investigate territorial male responses to differing song complexity levels.

My aim was to investigate breeding male tui responses to simple and complex song in intruding male tui. This was tested using playback experiments to gain understanding about the function of song complexity in the context of tui territoriality. As songbird song complexity plays a role in territory tenure, and female extra-pair mate selection (Potvin et al. 2015), intruding males with more complex songs are likely to be perceived as stronger competitors by territorial males. Theoretically, this would be particularly true in species with high extra-pair paternity who risk suffering cuckoldry in addition to losing territory. Therefore, I predicted more complex songs of intruding male tui would indicate a greater challenge to territorial males, and thus would elicit stronger, more aggressive responses than simpler song.

4.2 Methods

4.2.1 Locations

I conducted playback experiments at 12 locations in Ecology Bush, Tawharanui Regional Park (36°22’S, 174°50’E), 90 km north of central Auckland, New Zealand. Tawharanui is a 588-ha predator-fenced mainland island on the Tawharanui Peninsula (Figure 4.1). The park consists of grazed pasture and coastal native forest remnants where tui are abundant. Each playback location was conducted within a territory of a focal male.

Figure 4.1: Map showing the location of Tawharanui Regional Park and the 12 playback locations within the park. Playback locations 6, 8, 9, 10 had banded focal males.

The distance between adjacent playback locations was approximately 200 m. To test whether stimuli from a playback speaker (SAST PT06 Loudspeaker, Shenzhen SAST Electronics Co. Ltd, Shenzhen, China) could be detected by a non-target tui 200 m from the sound source, I recorded a randomly-selected complex song stimulus (see below for more details on the stimuli) from 200 m away (at 80 decibels relative to a carrier level [dBC] from 1 m, fast setting, measured using a sound level meter, Digitech QM-1589) using a long-range microphone that had a frequency response range of 40 Hz to 20 kHz (Sennheiser ME67, Sennheiser, Old Lyme, CT) attached to a Marantz PMD620 digital recorder (Marantz, Kanagawa, Japan). The stimulus however was undetectable on the spectrogram produced by Raven Pro software (1.4 Beta Version, Cornell Lab of Ornithology, Ithaca, NY, USA). Nevertheless, I conducted a playback of this recording to tui 20 m away from the speaker (at 80 dBC), which evoked no response. Therefore 200 m was used as the distance between playback locations to minimise the chance of tui from other playback locations detecting and habituating to the playback stimuli.

Of the 12 locations, four of the territorial male tui were banded and 8 unbanded. One was banded in 2011 and three in 2013. The breeding stage of two males were confirmed, one nest building and the other post-fledging. No other focal males had active nests that were found and were most likely in-between broods because the playbacks were conducted in mid-breeding season (on three separate days: December 11th, 14th and 18th 2014).

Tui are especially territorial in the breeding season and males sing regularly on specific perches within their territories (Bergquist & Craig 1988). Banded tui stayed within their territories throughout the duration of the playback experiments. I was therefore confident the same bird was at each location on subsequent days for unbanded males. I identified tui territory boundaries by watching flights and movements of tui. The presence of a territorial male tui does not necessarily equate to the territory holder having a mate or a nest.

4.2.2 Stimuli

The stimuli consisted of complex broadcast male tui song, simple broadcast tui song, and control heterospecific song (of a male grey warbler, *Gerygone igata*). Grey warbler song was selected as a control as they are a non-related songbird commonly occurring in the same location and habitat, and can be regularly heard. Variables used to measure within-complexity of song stimuli were: 1) number of syllables (the total number of syllables within each song, Boogert et al. 2008). A syllable is defined as either a continuous tracing on the spectrogram; or a tight group of identical sound units. I considered a syllable as such, if it was separated from other syllables by a visible temporal interval ≥ 90 ms, 2) syllable diversity (number of *different* syllables within each song, Garamszegi & Møller 2004, Boogert et al. 2008). Different syllables were identified and distinguished by their duration, spectral structure, and bandwidth (the difference between minimum and maximum frequencies), 3) syllable transitions (the number of times the bird switches from one syllable type to another, Sasahara et al. 2012), and 4) song duration (length of time from the beginning of the first syllable to the end of the terminal syllable of each chosen song, Boogert et al. 2008) (Figure 4.2). In total, there were six different songs within each stimulus type category (complex, simple, and control) (Figure 4.3; Table

4.1) as per the recommendations in Kroodsma (1990). The mean syllable diversity (23 ± 2) within the complex tui song stimuli was above the mean of the Tawharanui tui population (19 ± 9, Hill et al. 2013).

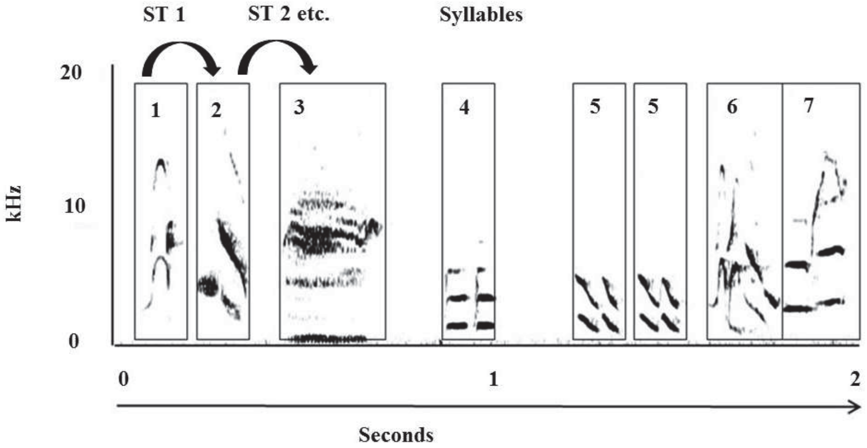


Figure 4.2: Spectrographic representation of total number of syllables, song duration, syllable diversity (total number of different syllables) and syllable transitions (ST) calculation. This two second tui song example has eight syllables in total, seven different syllables (as syllable 5 is repeated), and six syllable transitions.

Table 4.1: The mean complexity values ± standard error per song of the tui playback stimuli used in this study.

Variable Stimuli	Number of syllables	Syllable diversity	Syllable transitions	Syllable length (sec)	Song duration (sec)
Complex tui song	24 ± 2	23 ± 2	22 ± 2	0.24 ± 0.11	7.25 ± 1.03
Simple tui song	6 ± 1	6 ± 1	5 ± 1	0.17 ± 0.01	6.93 ± 1.97
Control song	6 ± 1	3 ± 1	3 ± 1	0.07 ± 0.04	8.89 ± 0.36

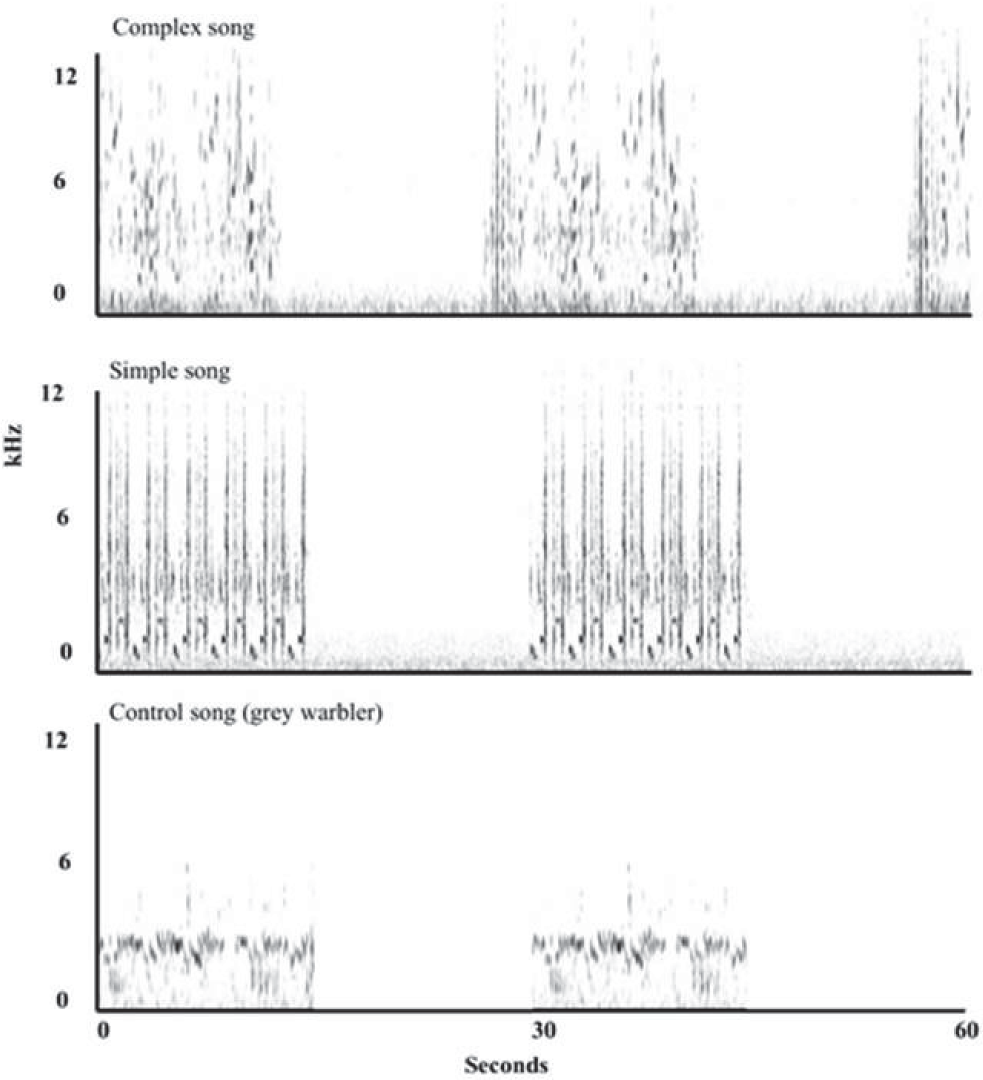


Figure 4.3: Sixty-second exemplars of the song stimuli with different levels of complexity (the stimuli): complex song, simple song and control heterospecific song (grey warbler) used in these playback experiments. Each period of signal represents a single song bout and each song stimulus had a concordant signal-to-silence ratio.

All tui song stimuli were recorded from a single territorial male (ID code: YG-YM, 123 g) to control for responses to individuals instead of song complexity. The stimuli were recorded near Jones Bay approximately 1.5 km from the nearest focal tui territory, so were likely to be unfamiliar to each focal male at each playback location. The grey warbler songs used as controls were recorded from the park approximately 1.5 km from the nearest focal tui territory so were also likely to be unfamiliar to each focal male. All recordings written as wav files were made from the previous breeding season (at a sampling rate of 44.1 kHz, with a resolution of 24 bits). This was conducted using both the long-range directional microphone, attached to the digital recorder both described above.

The recordings were edited and stimuli produced using Raven Pro (1.4 Beta Version). The sound level meter was used to ensure the sound was at the same volume for each stimulus (80 dBC at 1 m, fast setting). This represented natural singing volume as measured by a sound level meter. Each stimulus was a recording with a good signal-to-noise ratio. One different song from the source male was used for every different stimulus. Every stimulus had the same ratio of signal (i.e. song bouts) to silence over the entirety of the three min [90 sec song, 90 sec silence] at intervals to simulate natural song as best as possible. Each focal male therefore was exposed to the same amounts of stimuli whether complex, simple, or control song.

4.2.3 Playback experiment

For the playback experiment, the position of the speaker was generally on the periphery of each focal bird’s territory. This simulated a commonly observed type of territory invasion in tui, where a male tui will approach, loiter and sing within a safe distance from the territory owner. Prior to the playback experiments I set out flagging tape at measured distances (2, 5, 10, 15 m) from the playback speaker in to aid in estimation of distances. I also marked speaker location so the experiment could be replicated on subsequent days (as per Searcy et al. 1997). The speaker was placed approximately 2 m above the ground. I waited until the territorial male was present in its regular perching area before commencing the experiment. Typically, the male would arrive in less than five min, although occasionally the male was already on a known singing perch.

The directional speaker was always oriented towards the birds' regular singing perch within each territory to minimise the chances of disturbing birds on neighbouring territories. I placed the speaker a minimum of 20 m from each bird's regular singing perch before initiating playback. From observations, when rival tui intrude onto territories, they will either arrive and sing on a peripheral perch, directly approach the territory holder on its singing perch or fly through the territory. Performing playbacks at this distance generally simulated the former, whilst minimising human disturbance of the focal bird. Observers stood 9 m from the speaker, therefore 29 m from each bird's regular singing perch.

Songs were played back as wav files from an Emachines E443 laptop computer (Emachines, Irvine, California, USA). The laptop was attached to the speaker by a 9 m cable. The speaker had a frequency response of 200 Hz–13,000 Hz (± 1 dB), similar to natural tui signals. The playback procedure consisted of a three min pre-stimuli observation period to confirm focal males were exhibiting normal behaviour (e.g. perching or foraging) and that they were not stimulated by conspecific song or already engaged in territorial confrontation. This was followed by a three min period of randomised stimuli treatment (complex tui song, simple tui song, or control song) during which eight main response variables were recorded. A three min period of post-stimuli observation followed during which 5 of the 8 main response variables (refer to Table 6.2) were recorded from the focal male. Songs were shuffled and each song assigned a number (from 1-18, six songs within each of the three stimulus type categories). The ‘Random Number Generator’ app on an iPhone® (Apple, Cupertino, CA) was used to determine which song was to be played. It was ensured three different stimulus types (complex, simple, and control) were played at each location across the three playback days. On day two, a random number was generated for each location as previously but if it was a song that had already been played at that location on day one, another random number was generated to correspond with an unfamiliar song stimulus. I did the same on day three to ensure songs from all three stimulus types had been played at each location over the three days without any being repeated.

Table 4.2: Variables recorded as measures of the levels of aggressiveness of territorial male tui in response to the playback stimuli (Beecher & Campbell 2005, Parker et al. 2010).

Response variable	Stimulus stage recorded
Latency of first vocal response (sec)	During
Latency of first flight or movement (sec)	During
Distance from speaker after 30 sec (m) (for statistical purposes, any distance over 15 m was entered as ‘16‘ into the statistical program for all distance-related response variables)	During
Distance from speaker after 2 min (m)	During
Closest distance to speaker (m)	During and post
Total number of flights towards/over/in response to stimuli (total number of flights towards or aggressively away from speaker). Hops from branch to branch were not included in the count	During and post
Length of time within 2 m of speaker (sec)	During and post
Total number of songs	During and post
Complexity of vocal response of focal male (using 4 measures of complexity) – analysed separately from the other variables	During and post

During the playback experiment, I used three observers to record the response of the focal bird (two of whom were unaware of the hypothesis, i.e. blind). A fourth person acted as an observer to track the movement of the focal male should it fly out of sight of the three main observers. The observations from all three observers had to concur for that behavioural measure to be included. We had high between-observer agreement. Vocal responses of the focal males were simultaneously recorded using the long-range directional microphone attached to the digital recorder during and post-stimuli.

The intervals of three and four days between playback sessions respectively to each focal male minimised the chances of habituation to song stimuli. The playbacks were conducted between 0600 and 0930 during dry and still weather. Rain and wind gusts occurred on one occasion during the playback experiments but cleared within approximately 12 min. The playback was paused and continued once the weather was dry and still again.

For unbanded tui, it was possible to differentiate males from females, due to sexual dimorphism. Males are significantly larger on average than females (Wells et al. 2015). The sex and the number of individuals responding were noted during each playback session.

4.2.4 Statistical analysis

A principal component analysis (PCA) was used to combine the eight correlated measures of response into fewer orthogonal measures. The complexity measures of vocal responses were analysed separately (see below). Three values per individual, per variable, were entered into the model as independent datapoints (complex, simple, and control). Data were normalised using log transformations to ensure even standard deviations across the variables. PCA is useful in playback experiment analyses, as it makes the interpretation of responses to playback easier and avoids the statistical issues concerned with correlated measures. Post-hoc tests were run to determine significant differences between the different combinations of related groups. Firstly, a Friedman's test was used to detect differences in the strength of responses between treatments (i.e. stimuli) across multiple test attempts. The data were paired, so a post-hoc Wilcoxon matched-pairs signed rank test with Bonferroni correction was used to identify significant differences in the aggressiveness of response to the stimuli for each of the response variables (one at a time) in a pairwise mode. Wilcoxon tests were conducted on the different combinations of groups (complex, simple and control song). I also compared the resulting PCA scores using Wilcoxon matched-pairs signed rank tests between treatments.

To further test the strength of responses from the three stimuli, the raw response variables were examined by analysis of similarities (ANOSIM) following standardisation to Z-scores (i.e. normalisation). ANOSIM is a non-parametric multivariate test based on Euclidean distance, using the rank order of dissimilarity values to test whether the strength of responses (of the 8 main response variables) differed significantly between the three stimuli. A one-way permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) based on Euclidean distance was also used to test whether the strength of responses differed significantly between the three stimuli types. PERMANOVA was run on matrices of Euclidean dissimilarity calculated from the transformed and standardised data. It partitioned sources of variation with methodology analogous to ANOVA and used unrestricted permutations of raw data to test for statistical significance in the data (Anderson 2001, Olabarria et al. 2010). Multivariate PERMANOVA is highly robust to correlations and heterogeneous variances (Anderson & Walsh 2013).

4.2.5 Complexity measures of vocal responses

A single experienced observer (the author) used Raven Pro to collect four measures of within-song complexity from the vocal responses to stimuli: number of syllables per song, syllable diversity per song, syllable transitions per song, and song duration (as described in the 'stimuli' subsection). Some songs after being analysed were re-evaluated to maximise consistency. Not all focal males responded vocally to the song stimuli during the playback experiment. Song complexity therefore was not included in the analysis alongside the other eight response variables because song complexity can only be measured when vocalisations are produced. Instead, I used one-way ANOSIM and PERMANOVA tests to compare complexity of any vocal responses from focal males in response to the different stimuli. A mean of three response songs were analysed for every focal male that responded with songs.

The ANOSIM and PERMANOVA were performed using PAST software (version 2.17b) (Hammer et al. 2001). The ANOSIM and PERMANOVA tests were conducted with 9999 permutations, and with the Bonferroni correction for multiple comparisons. All other statistical procedures were conducted using SPSS (version 20.0, IBM, Chicago, IL, USA).

4.3 Results

In 19 of the 24 tui song playback sessions (79% of the total number of tui song playbacks), only males responded. Females responded, physically but non-vocally, to both complex song on four occasions, and simple song on one occasion. On these occasions, females approached the focal male non-aggressively and perched alongside the focal male. More than one individual responded in 14 tui song playback sessions (58%), and in nine of these 14 sessions (64%), the responses were exclusively to the complex song stimuli. In five of these 14 sessions (36%), males responded to both complex *and* simple stimuli. It is possible that other individuals aside from the focal bird responded to both the song stimuli and subsequent vocalisations of the focal male. Only the focal male's response measurements however were included in the statistical analysis.

The PCA for the response variables showed that PC1 explained 71.69% of the variance between the three groups, whereas PC2 explained 12.37% (Table 4.3). A Friedman's test on the raw response variable values for each individual revealed that there were significant differences in the strength of response across the three different stimuli ($N = 12$; $\chi^2_2 = 19.5$; *df*: 2; $P < 0.001$), i.e. this allowed us to reject the null hypothesis that there were no differences in the strength of response for all eight main variables to all three stimuli. There were also significant differences in PC1 scores between treatments (Table 4.4). Coupled with the scatterplot (Figure

4.4), this also suggests there were differences in the strength of response of the focal birds to the three different stimuli.

The PCA eigenvalues suggested seven of the variables were correlated (Table 4.3). A post-hoc Wilcoxon matched-pairs signed ranks test (with Bonferroni correction) was conducted on the variable that contributed the most to the variation between the groups on the first PC (closest distance to speaker [m]). This variable essentially represented these seven variables measuring the aggressiveness of response. The variable contributing the highest variance on PC2 was also selected for further analysis (total number songs produced during and post-stimulus), even though PC2 contributed little to the variance between the groups (Figure 4.4). The two variables that contributed the most to the variation between the different stimuli on PC1 and PC2 respectively were: 1. The closest distance to speaker, and 2. The total number of songs produced during/post-stimuli (total of six min). Responses of all variables to the complex song stimuli were stronger than simple and control song stimuli, suggesting complex song evoked more aggressive responses from the focal males within their territory (Table 4.3).

One-way ANOSIM and PERMANOVA tests showed a significantly stronger response of territorial males to complex song than simple (ANOSIM: N = 12; $R = 0.385$; $P < 0.001$, PERMANOVA: *Pseudo* $F_{3,\,12} = 7.325$; $P = 0.002$) and control song (ANOSIM: $R = 0.995$; $P < 0.001$, PERMANOVA: *Pseudo* $F_{3,\,12} = 83.35$; $P < 0.001$) suggesting complex song stimuli evoked more aggressive responses from focal males than simple or control song.

Table 4.3: Eigenvalues, variance explained and factor loadings of the response variables following principal component analysis (PCA) for the response of focal males to complex, simple or control stimuli. *Variables with factor loading values greater than 0.7.

	PC1	PC2
Eigenvalue	5.73	0.99
% of variance	71.69	12.37
Latency of first response to stimuli (sec)	0.91*	0.30
Latency of first flight or movement towards the speaker (sec)	0.91*	0.29
Distance from speaker after 30 sec (m)	0.88*	-0.06
Distance from speaker after 2 min (m)	0.84*	-0.32
Closest distance to speaker (m)	0.95*	0.12
Total number of flights towards/over/in response to stimuli	0.83*	-0.37
Length of time within 2m of speaker (sec)	-0.63	0.71*
Total number of songs during and post-stimuli	-0.78*	0.24

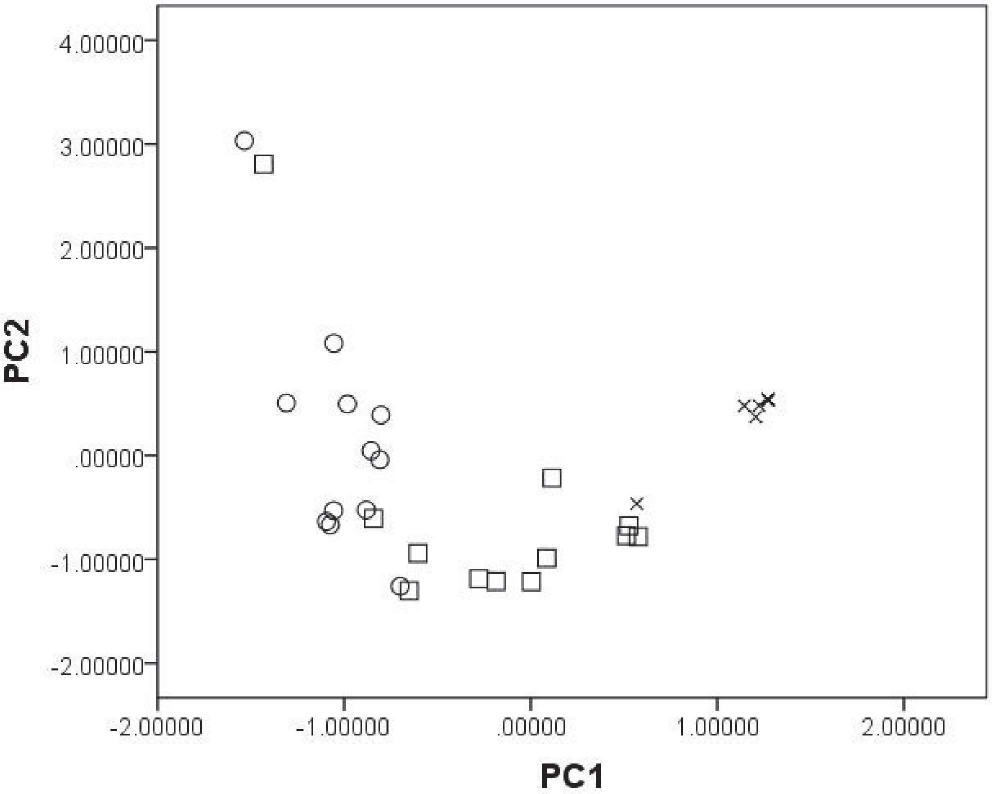


Figure 4.4: PCA scatterplot with Eigenvalue scales along the axes (circles: complex song, squares: simple song, crosses: control heterospecific song).

Table 4.4: The descriptive statistics including the means ± standard error values and *P* values from the Wilcoxon matched-pairs signed ranks test of the two variables that contributed the most variance in the PCA for all three stimuli types. Also shown are the mean PC1 Eigenvalue scores for each treatment. Note that only the *P* values for complex versus simple song are shown (N = 12).

Variable	Control song	Complex song	Simple song	*Z*	*P*
Closest distance to speaker (m)	15.1 ± 0.9	0.3 ± 0.2	6.3 ± 1.6	-2.807	0.005
Total number of songs during and post-stimuli	1 ± 0	15 ± 3	7 ± 2	-1.649	0.099
PC1 Eigenvalue score	1.2 ± 0.1	-1 ± 0.1	-0.2 ± 0.2	-2.824	0.005

4.3.1 Complexity of vocal responses

The complexity values of vocal responses were averaged for each individual before conducting multivariate analyses. Males had a significantly higher response to complex song than to simple song for all four song complexity variables (ANOSIM: N = 6, 8, *R* = 0.5092, *P* = 0.005, PERMANOVA: *Pseudo* $F_{9, 14}$ = 11.44; *P* = 0.005, Table 4.5; Figure 4.5).

Table 4.5: The means ± standard error of male song complexity variables in response to playback of complex and simple songs.

Stimuli	Complex song		Simple song	
Song complexity response variable	Per song	Per sec	Per song	Per sec
Number of syllables	22 ± 33.2	3 ± 0.44	8 ± 2	2.18 ± 1
Syllable diversity	21 ± 3	3.08 ± 0.44	9 ± 2	2.45 ± 1
Syllable transitions	19 ± 3	2.79 ± 0.44	7 ± 2	1.91 ± 1
Song duration (sec)	6.81 ± 1.34	-	3.67 ± 0.81	-

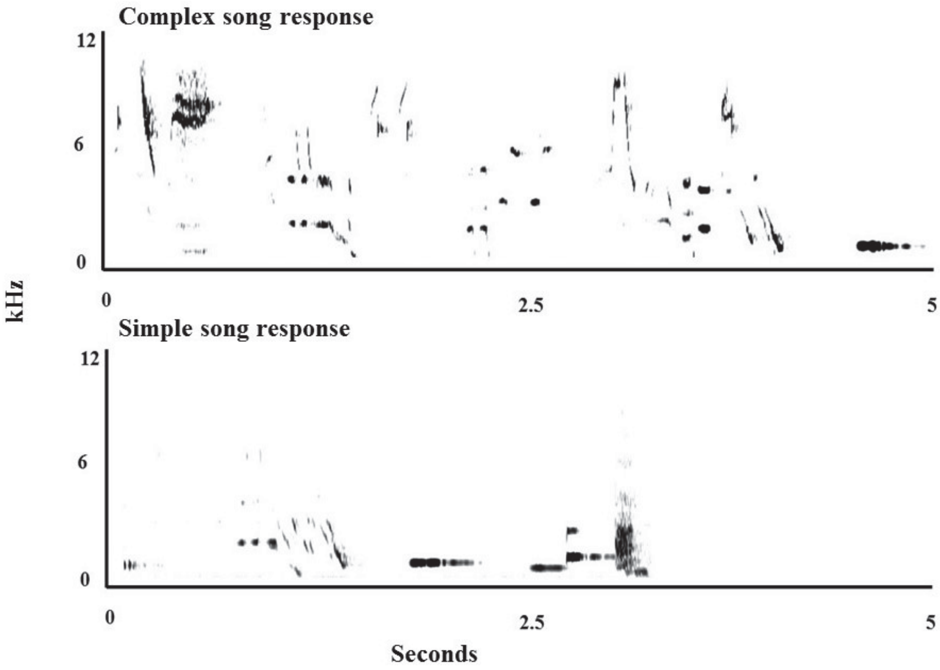


Figure 4.5: An exemplar of tui song responses to complex song and simple song playback.

4.4 Discussion

As predicted, complex song stimuli evoked significantly stronger responses from territorial males than simple song stimuli. Although all focal males did respond to both song types, the responses were more aggressive to the *complex* song playback stimuli. In addition, complex songs evoked more complex vocal responses from focal males. During complex song playback sessions, males would generally remain close to the speaker for the duration of the playback stimuli and for the entire three min post-stimuli period. This suggests that the complex song stimulated both a greater initial aggressive response and a longer lasting aggression from focal males.

Complex song is seemingly perceived as a greater threat to territorial males than simple song. It may provide information about the signaller such as his quality, aggression, and degree of territoriality, in addition to his intentions, and level of attractiveness to females. These information types could be used by territorial males to determine the threat significance of intruding males. Results from this experiment suggest birds will more strongly interact both physically and vocally with the speaker when it emitted complex song stimuli. In some songbird species, song matching tends to play a key role in territory defence (Searcy & Andersson 1986; Catchpole & Slater 2008). Males sang more complex songs in response to more complex stimuli, although no matching of syllable types was observed. While exact song matching did not occur in this study (the songs were equally unfamiliar to all focal males), focal males sang for longer and to highly complex songs presumably to match the complexity of the songs emitted by the speaker. Indeed, the data suggest that focal males responded to complex song stimuli with longer and more complex songs than to simple song stimuli. This suggests that responsive singing functions to transfer information to an intruder about its motivation or aggression.

Singing more complex songs has been linked with males obtaining more extra-pair fertilisations in other songbird species (Hasselquist et al. 1996). In tui, a species with high extra-pair paternity frequency (57%, Wells et al. 2015), complex song is likely perceived as a threat to territorial males due to the possibility of extra-pair copulations of the intruder with a local female. There would be a steep drop in a territory holder's fitness if his partner increased extra-pair copulations (Kokko 1999; Eliassen & Jørgensen 2014), hence rigorous territoriality is often required to mitigate this risk.

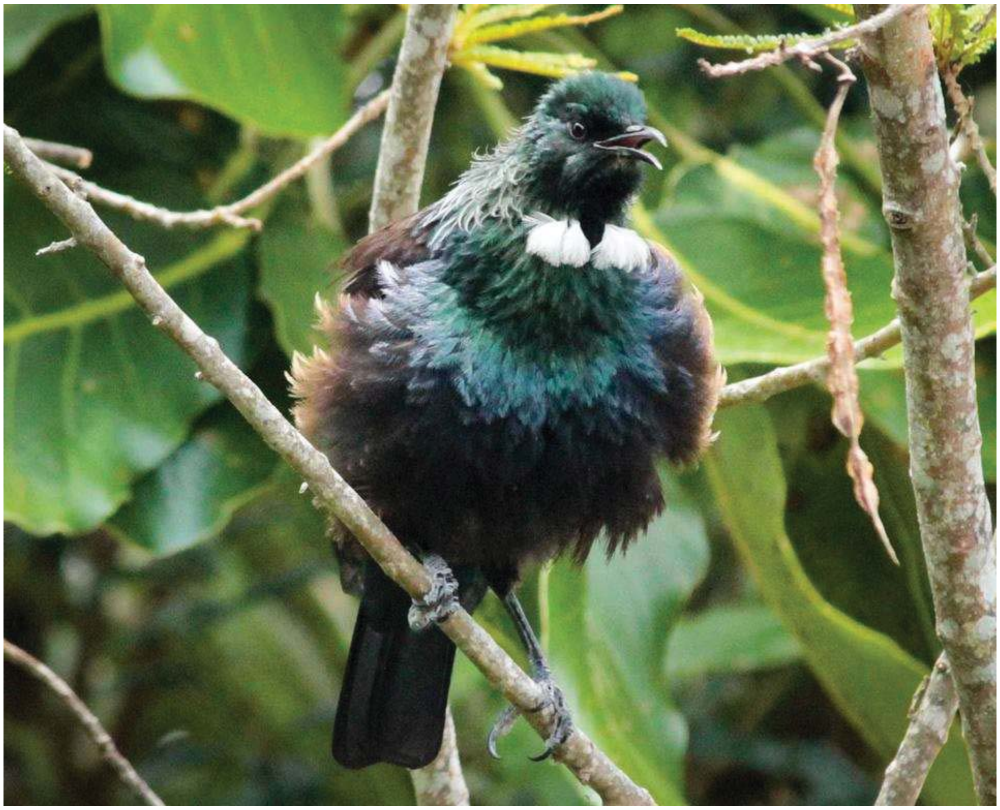
Observationally, the playback of complex song stimuli during the trials induced a greater deal of physical aggression between responding males. This gives further support to the role complex songs play in territorial encounters in songbirds. In 58% of cases, more than one male responded to the song stimuli. Most responses of non-focal males were to complex song stimuli. Evidence suggests that longer, more complex songs function mainly for female attraction (Catchpole and Slater 2008) but also male-male territoriality (McGregor & Horn 1992). It is likely therefore that mate attraction and rival repulsion are intrinsically linked, because a male that sings more complex songs is more likely to obtain a partner and is therefore a tangible threat to any territorial male (Seddon et al. 2013).

An individual’s level of aggression and fighting ability can reflect its physical performance capacities and its physiological state (Lailvaux & Irschick 2006). Aggressive encounters in the natural world can be highly ritualised or result in actual fighting (Alonso-Alvarez et al. 2004). It has been shown that females will also assess a male’s potential physical fighting ability through a well-choreographed display when selecting a partner (Alonso-Alvarez et al. 2004) because fighting ability could reflect overall quality of the male, although the extent to which this occurs in tui is unknown. Indeed, many male songbirds use song and song matching as their main means of advertisement of quality to rival males and potential mates (Kroodsma 1979).

In this study, complex song evoked strong male responses with a limited amount of female response. It must be acknowledged that using songs from a single male may limit the conclusions that can be drawn from this study. For example, unknown and undetected characteristics of the male used to obtain the stimuli may have elicited certain response types. Future studies in this area should therefore consider these factors in the experimental design phase. Further studies examining how complex song varies with reproductive success of males and females in addition to male condition are recommended to establish whether song complexity is an indicator of tui male reproductive quality. Furthermore, the effect of song on females and the role of complex song in mate attraction in complex vocal species needs further consideration.

In summary, the results indicated that territorial male tui responded more strongly to complex song than simple song stimuli during the breeding season. Complex song was perceived as a threat and appeared to act as a trigger for aggressive male-male encounters. My findings set a platform for future studies on the relationship between tui song complexity and both reproductive potential and male quality, measured in terms of neural constraints, genetic diversity, developmental stress levels and parasite loads.

5 Local habitat complexity correlates with song complexity in a vocally elaborate honeyeater



A singing male tui (photo: Jon-Paul Hansen, with permission).

This chapter was published in *Austral Ecology* (Appendix 1).

Abstract

Song complexity is an important behavioural trait in songbirds, subject to sexual selection. Elucidation of intraspecific variation in song complexity can provide insights into its evolution. In this study, I investigated song complexity variation in tui (*Prosthemadera novaeseelandiae*), a vocally complex songbird endemic to New Zealand. At two separate nature reserves, we recorded male songs in two habitat types: forest remnants with high habitat complexity, and open habitats with lower habitat complexity. Analyses indicated strong evidence that song complexity was higher in forest habitats. Possible explanations for this divergence include: (i) competition between individuals results in higher quality, dominant males with more complex songs occupying forest habitats, and less competitive males occupying open habitat zones; (ii) forest habitats provide more abundant resources therefore higher tui density, resulting in more complex songs; and (iii) a higher abundance of food in dense forest habitats may reduce nutritional stress during development resulting in full development of song nuclei. However, these hypotheses on the drivers of habitat effects on tui song complexity remain to be tested.

Keywords: habitat complexity, singing behaviour, songbird, song complexity, tui

5.1 Introduction

In songbirds, the principal functions of song are territory defence (De Kort et al. 2009) and mate attraction (Briefer et al. 2010). Understanding ecological factors that influence intraspecific variation in sexually selected traits, such as song complexity, can provide important evolutionary insights.

Complex songs are costly to produce (Searcy 1992). Evidence suggests song complexity is a proxy for male fitness and a product of sexual selection and intrasexual competition (Spencer et al. 2004; Spencer & MacDougall-Shackleton 2011). Varying degrees of song complexity was found to influence male mating success (Hasselquist et al. 1996). Males with more complex songs tend to establish breeding territories earlier than males that produce less complex songs (Alatalo et al. 1986).

Song complexity can be measured using several variables such as syllable diversity (Grunst et al. 2016), song length (Gil & Gahr 2002), and trill proportion (Leitão et al. 2006). Trills are honest signals of male quality (Hennin et al. 2009). Both trills and songs are longer in closed and dense habitats (Wiley 1991; Van Dongen & Mulder 2006). Acoustic adaptation to divergent habitats can influence intraspecific communication and generate phenotypic variation in song complexity characteristics (Handford & Nottebohm 1978; Tubaro et al. 1993). However, there is no strong evidence that other song complexity measures such as syllable diversity and entropy vary in different habitats.

Tui (*Prosthemadera novaeseelandiae*) are endemic New Zealand songbirds within the Meliphagidae (honeyeaters). They exhibit sexual size dimorphism and have high extrapair paternity (Wells et al. 2015). Natal philopatry and limited dispersal also occur in tui (Bergquist 1985; Stewart & Craig 1985). This species is a good model for investigating intraspecific variation in song complexity since they colonise various habitat types and produce complex vocalisations (Hill et al. 2013; Hill et al. 2015). Previous studies found that mainland tui songs were more complex than on remote islands (Hill et al. 2013). There is also evidence for differences in tui song phrases at the microgeographic level (Hill & Ji 2013). However, whether tui song complexity varies at the microgeographic level is still open to speculation. Using tui as a model, within two nature reserves, I compared song complexity within two local habitat types with divergent levels of habitat complexity: (i) native forest fragments with more complex plant communities (forest); and (ii) habitat zones consisting of pasture and sporadic trees (open). I predicted higher song complexity in forest habitats than in open habitats, giving rise to microgeographic song complexity variation.

5.2 Methods

5.2.1 Study sites

Tui song recordings were collected from both closed forest remnants and open habitats, at 2 rural study sites in northern New Zealand: Tawharanui Regional Park and Wenderholm Regional Park (Figure 5.1).

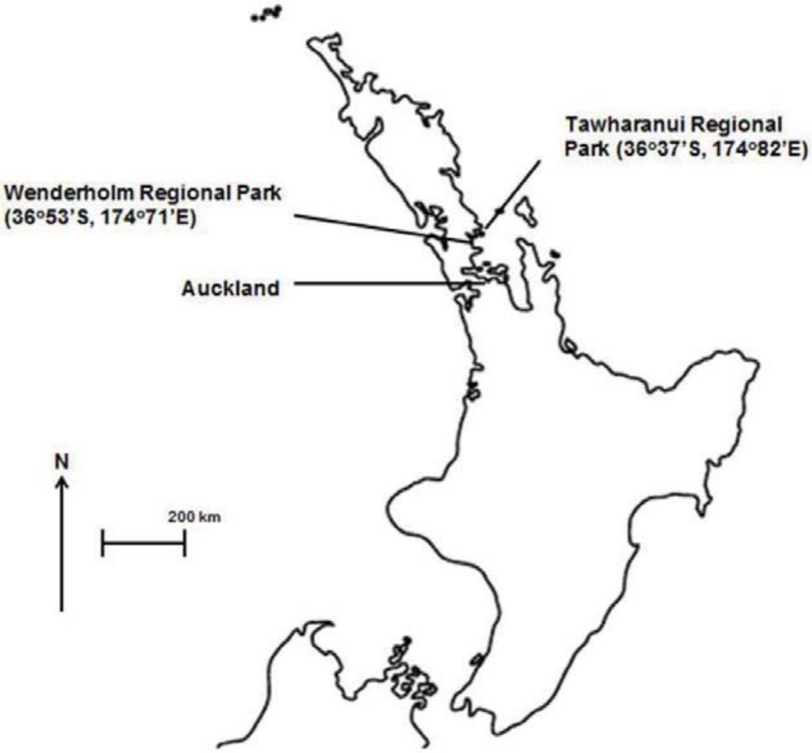


Figure 5.1: Map showing the location of the two study sites: Tawharanui and Wenderholm Regional Parks.

Tawharanui is a 588 ha predator-free nature reserve 90 km north of central Auckland, New Zealand. The forest site here, Ecology Bush (36º37'S, 174º84'E; approximately 70 ha), consists predominantly of mature coastal forest remnants. Tree species include important food resources for tui (e.g., puriri *Vitex lucens*, rewarewa *Knightia excelsa* and tawa *Beilschmiedia tawa*) with patches of regenerating scrub such as kanuka (*Kunzea robusta*, Hill et al. 2013). The open habitat study site at Jones Bay (36º37'S, 174º82'E; approximately 59 ha), approximately 2 km from Ecology Bush, consists of large open pasture with small patches or isolated trees. Important food plant species for tui in the open habitat include native trees (e.g., kanuka, karo *Pittosporum crassifolium* and pohutukawa *Metrosideros excelsa*), and exotic species such as flame trees (*Erythrina x sykesii*).

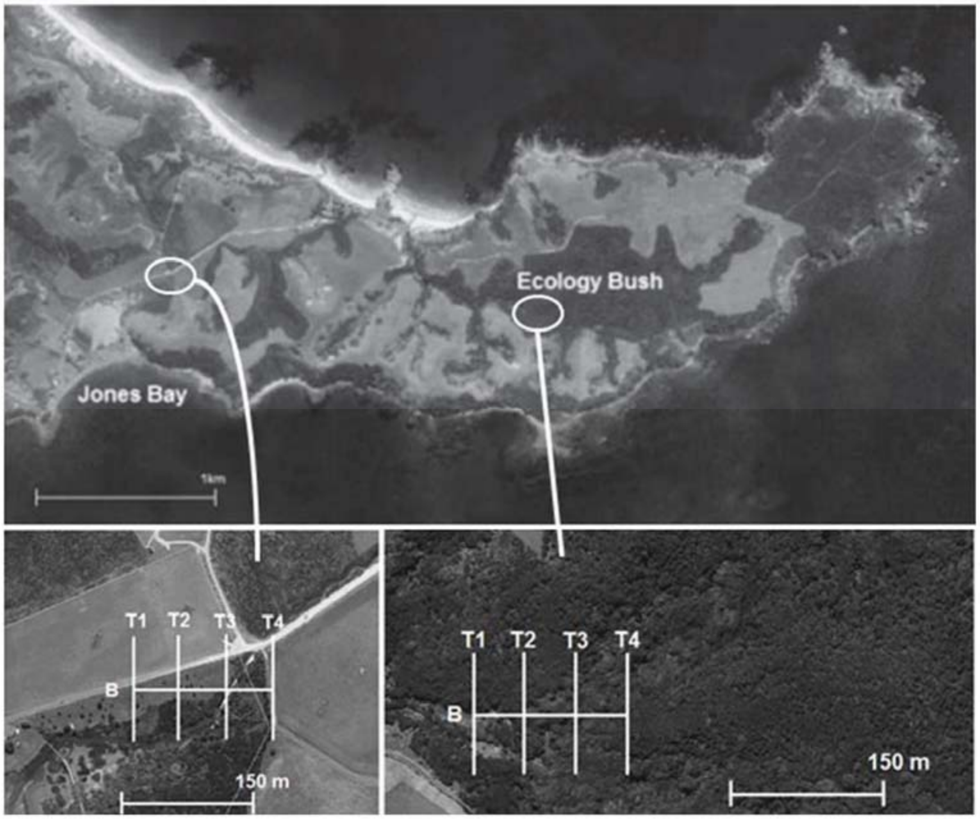
Wenderholm is a 134 ha predator-controlled nature reserve 46 km north of central Auckland, New Zealand. The park sits bounded by two tidal estuaries with an 80 ha hilly forested headland. The two sampled habitats at Wenderholm lay approximately 1 km apart. The forest area [36º54'S, 174º71'E] features important food resource species for tui such as kanono (*Coprosma grandifolia*), karamu (*Coprosma robusta*), and totara (*Podocarpus totara*). The open habitat [36º53'S, 174º71'E]) predominantly contains tui food resource species such as houpapa (*Pseudopanax lessonii*), karo and pohutukawa.

5.2.2 Data analysis

Habitat complexity

To quantify habitat complexity, I measured vegetation structure and plant diversity. At each site, I conducted vegetation surveys along 4 x 140 m transects (on pre-determined bearings using a compass, Figure 5.2), using the point-centred quarter (PCQ) method (Cottam & Curtis 1956), with 20 m intervals between centres of PCQ survey points. At each sampling point, a 'cross' using 2 x 1 m aluminium tubes was placed on the ground to create four quarters. At each quarter, the tree or shrub with a diameter at breast height (DBH, 1 m 35 cm) of ≥ 7.85 cm (2.5 π cm, measured using diameter tape) closest to the centre PCQ point was selected to measure. Established forestry protocol states that trees ≤ 7.85 cm are considered saplings (e.g., Vargas-Rodriguez et al. 2005). The plant species, DBH and the distance from the quarter to the centre point of the tree were recorded.

(a)



(b)

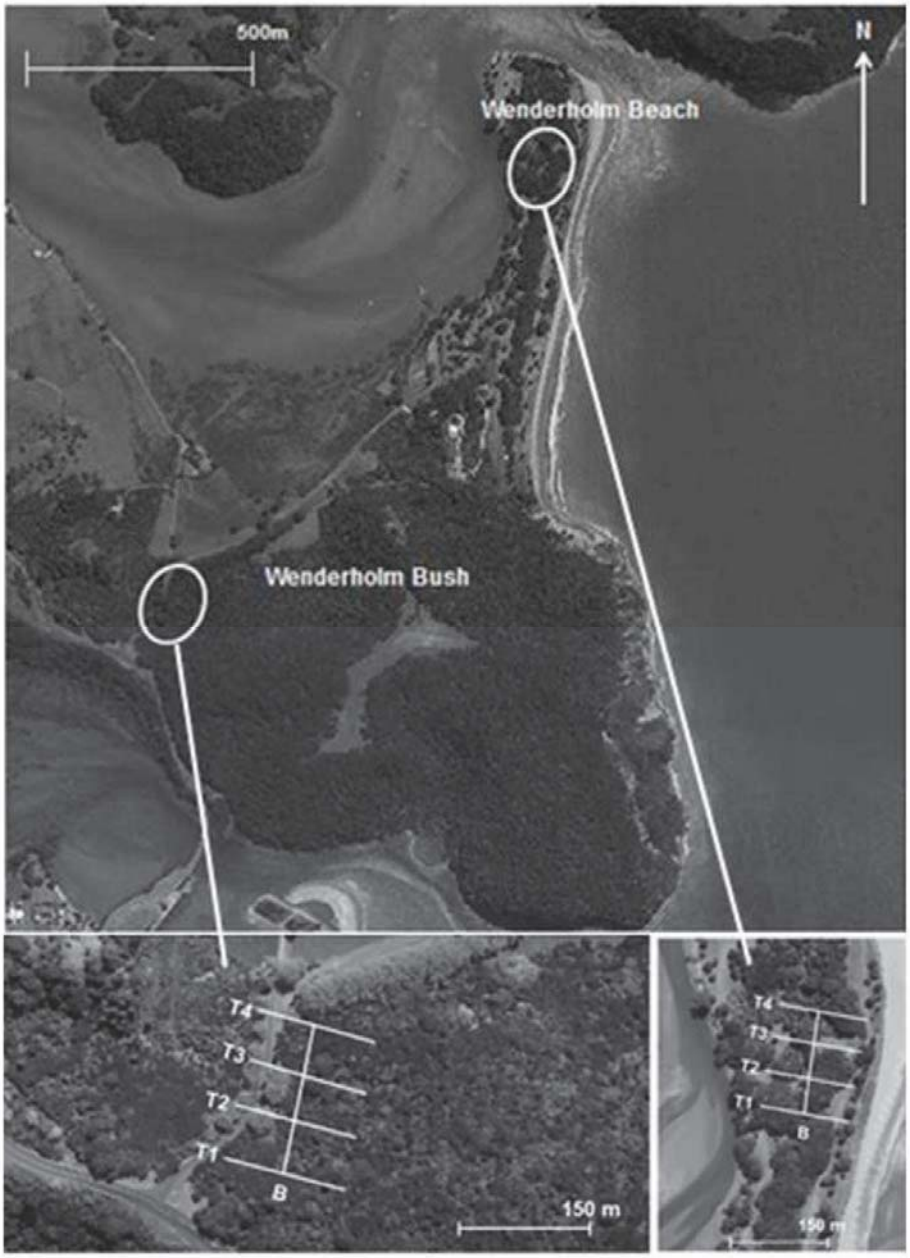


Figure 5.2: Maps showing the location of the habitat complexity survey sites plus the transect lines (T) and bird recording transect lines (B) at Tawharanui (a) and Wenderholm Regional Park (b).

Three variables were employed to measure habitat complexity: (i) distance between the plant measured and the centre of the PCQ, which gave a measure of plant density; (ii) plant species richness, the total number of species recorded during the PCQ survey at each habitat (Gotelli & Colwell 2010); and (iii) DBH, measured for each individual tree, which is an indicator of tree size and maturity. Numerical habitat complexity data were normalised using a Z-score transformation. A one-way multivariate permutational analysis of variance (PERMANOVA) (Anderson et al. 2001) using Euclidean distance and 9999 permutations was conducted to examine differences in habitat density between the forest and open habitat zones at both nature reserves.

5.2.3 Sound recordings

A Marantz (Marantz, Kanagawa, Japan) PMD620 solid-state digital recorder was used to record songs along with a Sennheiser (Sennheiser, Old Lyme, CT) ME67 shotgun long-range ultradirectional microphone. Ultradirectional microphones are advantageous over omnidirectional microphones for recording focal individuals as they permit the procurement of high-quality recordings with minimal peripheral noise. All songs were recorded at a sampling frequency of 44.1 kHz, and at a resolution of 24-bits. The frequency response range of the long-range microphone was 40 Hz to 20 kHz.

For each habitat, I recorded adult male songs over a full day during tui breeding season (in October 2012) between 0550 hr (one hr before official sunrise) and 2100 hr (one hr after official sunset). Recordings were made in calm and dry climatic conditions. Recording days were divided into three separate 3 hr recording sessions: dawn (starting recording 1 hr before official sunrise until 2 hr after official sunrise), solar noon (starting recording 1.5 hr before official solar noon until 1.5 hr after official solar noon), and dusk (starting recording 2 hr before official sunset until 1 hr after official sunset). This ensured a standardised 9 hr of recording effort in each habitat. During recording sessions, territorial tui were located along a pre-determined 150 m line in each habitat. This line bisected the habitat survey transect lines described above. When a tui was located, I initiated recording. Recording was terminated when the tui ceased singing or vacated its singing perch. During each recording the location, time, unique band combination (if applicable), behaviour and song type of tui were documented. For unbanded birds, only one individual was recorded during any one recording bout (i.e. when the individual was singing). Individuals generally were recorded more than once throughout each day. During the breeding season when tui rigorously defend nests, breeding partners, and flowering plants, males have a propensity to be bound to their territories (Hill et al. 2015). Observations of colour-banded tui suggest some individual males will occupy the same territories for at least three successive breeding seasons (S. D. Hill, personal observation, 2015; S. J. Wells, personal communication, 2015). Territories were identified to the best of my ability. Resident males actively expelled intruding males from their territories. Although the possibility of male tui moving between sampling locations during the breeding season cannot be excluded, I expect the likelihood to be low.

The recordings were made at a distance of between 2 m and 10 m from each focal bird. A song was defined as a continuous singing sequence with a silent period of ≥ 3 s before and after another singing sequence (e.g., Woolley & Rubel 1997). Only song recordings with good signal-to-noise ratios were analysed (≥10 db). If significant background noise from vocalisations, climatic factors or other sources were present on a song spectrogram, it was not included in the analysis. Furthermore, low-frequency background noise (≤ 0.4 kHz) was excluded from every song analysed. This was done without clipping any focal bird's signal on the recordings (therefore, no song measurements were affected). At Tawharanui, nine different individual tui were recorded in Ecology Bush and seven at Jones Bay (16 in total). At Wenderholm, six tui were recorded in the Wenderholm forested area and five in the Wenderholm open habitat (11 in total) (Table 5.1).

Table 5.1: The number of recorded birds, the number of songs recorded (625 songs in total), mean songs recorded per individual and the sampling effort in each habitat.

	Ecology Bush	Jones Bay	Wenderholm forest	Wenderholm open
Total number of birds recorded	9	7	6	5
Total number of songs analysed	166	251	104	104
Mean songs per individual (± SE)	18 ± 3	36 ± 9	17 ± 5	21 ± 4
Total recording effort (hr)	9	9	9	9

Twelve of the 16 recorded tui at Tawharanui were uniquely colour-banded but no tui were banded at Wenderholm. In total, 625 tui songs were included in the analyses across the two nature reserves (Table 5.1). Both male and female tui produce songs, however females sing far less frequently. Tui produce two main types of song. Firstly, broadcast songs, vocalised from an elevated singing post to no obvious conspecific audience (Hill et al. 2013). Secondly, interactive songs that are produced when another tui is in proximity. Male tui broadcast song is the most frequent song type produced and can generally be recorded for every singing male. Female and male interactive songs on the other hand are significantly less frequent and cannot necessarily be sampled during recording sessions. There is evidence that broadcast and interactive songs also structurally differ (S. Hill, unpublished data, 2016). Consequently, only male broadcast songs were included in the analysis. The song spectrograms were digitised and variables measured using the Raven Pro 1.4 Beta software (Cornell Lab of Ornithology, Ithaca, NY, USA). For all recordings, spectrograms were created by Discrete Fourier Transform (DFT) with a Hann window. The frame length was 256 points, and I used a 50% frame overlap with a hop size of 2.9 ms, frequency grid spacing of 172 Hz, and the bandwidth was set at 3 dB. Signal-to-noise ratios were measured on Raven Pro using the 'band limited energy detector' function.

5.2.4 Comparison of song complexity between habitats

Five song complexity variables (Figure 5.3; Table 5.2) were extracted from each broadcast song recorded using Raven Pro. I averaged song variables for each individual tui for dawn, solar noon and dusk songs. Three individuals produced songs at two times of day (two individuals during dawn and dusk; and one individual during solar noon and dusk), and 24 individuals at just one time of day (12 individuals during dawn; nine during solar noon; and three during dusk), resulting in 30 different ‘individual-by-time’ combinations. We used a canonical analysis of principal coordinates (CAP) (Anderson & Willis 2003) to determine whether the five variables measuring song complexity were sufficient to discriminate between songs from forest and open habitats. The CAP was run in PRIMER v.6 (Clarke & Gorley 2006) using the 30 averaged values (described above). A ‘leave-one-out’ cross-validation assessed the discriminatory power of the model. We calculated the leave-one-out cross-validation results by removing a single individual-by-time averaged song from the data, and classifying its habitat using only its song complexity characteristics. We repeated this process for every song and the proportion of songs that were misclassified was calculated.

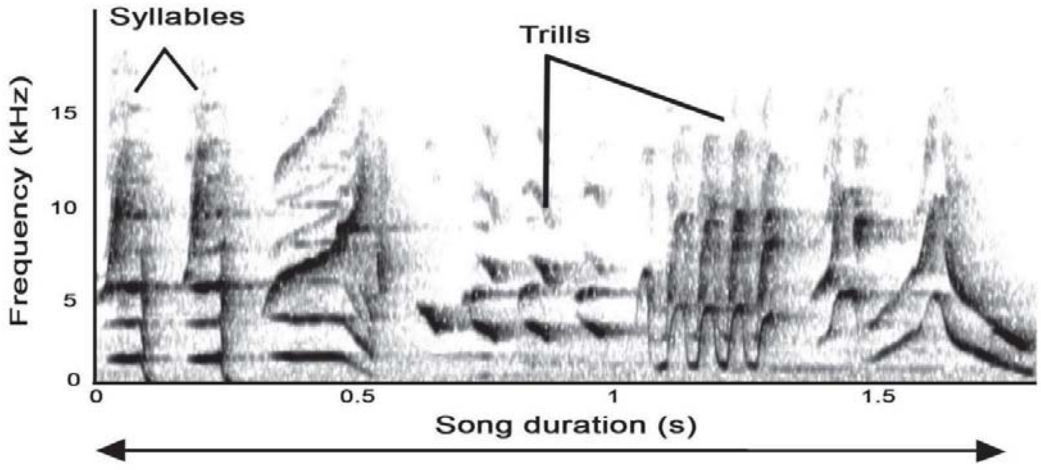


Figure 5.3: A spectrographic representation of the variables employed in this study to measure song complexity in tui.

Table 5.2: Variables employed in this study to measure song complexity.

Variable	Definition	References
Syllable diversity	Number of different syllables per song	Grunst et al. 2016
Song duration (s)	The length of song	Gil & Gahr 2002
Trill length (s) and trill percentage	The length, and proportion, of song that contains high-frequency trill syllables	Brumm & Slater 2006
Entropy	A unitless measure of song disorder/unpredictability. It measures spectral complexity. White noise, for example, has a high entropy score, a pure tone has a low entropy score $H_{sel} = \sum_{f=f_1}^{f_2} \left(\frac{E_{bin}}{E_{sel}} . \log_2 \left(\frac{E_{bin}}{E_{sel}} \right) \right)$	Charif et al. 2010

I used a one-way PERMANOVA using the Euclidean distance measure to test if there was evidence of a difference in song complexity between the forest and open habitats, using the same five song complexity measures. Values for each individual were averaged for the PERMANOVA as per the CAP analysis, with 9999 permutations. The song complexity values were averaged as per the CAP analysis.

PERMANOVA tests in this study were conducted using the PERMANOVA+ add-on package for PRIMER.

5.3 Results

5.3.1 Habitat complexity

The three variables of habitat complexity: distance of nearest tree or shrub from PCQ, plant species richness, and DBH at each PCQ, were significantly dissimilar between forest habitats and open habitats at both Tawharanui and Wenderholm (PERMANOVA: $N_{forest} = 512$, $N_{open} = 512$; *Pseudo* $F_{1020,\ 1024} = 128.9$; $P < 0.001$). Overall, habitat structure was more complex in the forest habitats including a higher density of plants with a DBH ≥ 7.85 cm, and greater plant species richness than the open habitats (Table 5.3).

Table 5.3: The habitat complexity descriptive statistics of two habitats at Tawharanui and Wenderholm. The number of trees and shrubs in each habitat relative to each PCQ is also presented.

Measurement	Ecology Bush (Forest)	Jones Bay (open)	Wenderholm forest	Wenderholm open
Mean distance (± SE) from PCQ to nearest tree/shrub (cm)	439.71 ± 41.5	1263.12 ± 50.12	409.3 ± 19.16	1443.02 ± 41.5
Total plant species richness	24	8	24	9
Mean (± SE) DBH of tree (cm)	38.32 ± 5.84	9.64 ± 1.57	23.24 ± 2.42	50.35 ± 5.49
Number of trees with a DBH > 7.35 cm within 20 m from each PCQ	113	64	128	69
Total number of trees and shrubs within 20 m from each PCQ (total: 256)	236	142	256	137

5.3.2 Song complexity

For both Tawharanui and Wenderholm, the overall misclassification error was 6.7%. This means the CAP model chose the correct habitat in 93.3% of songs analysed (Figure 5.4). This strongly implied that song complexity variation between the two habitats existed at both nature reserves. Song complexity was significantly higher in the forest habitats at both locations using all five song complexity variables (PERMANOVA: *Pseudo* $F_{21, 27} = 8.417$; $P = 0.002$) (Table 5.4).

Table 5.4: The descriptive statistics of the five variables measuring song complexity in two tui subpopulations at Tawharanui Regional Park and Wenderholm Regional Park.

Variable	Mean ± SE		Mean ± SE	
	Ecology Bush	Jones Bay	Wenderholm forest	Wenderholm open
Syllable diversity	18.85 ± 0.91	16.1 ± 0.52	19.67 ± 1.33	17.1 ± 0.93
Song duration (s)	11.36 ± 0.78	6.23 ± 0.27	8.28 ± 0.78	6.68 ± 0.52
Trill length (s)	3.20 ± 0.21	1.45 ± 0.07	2.38 ± 1.79	1.76 ± 1.11
Trill percentage	31.19 ± 1.79	24.39 ± 1.1	32.33 ± 1.18	30.43 ± 1.13
Entropy (average)	3.43 ± 0.35	3.13 ± 0.29	3.89 ± 0.47	3.30 ± 0.24

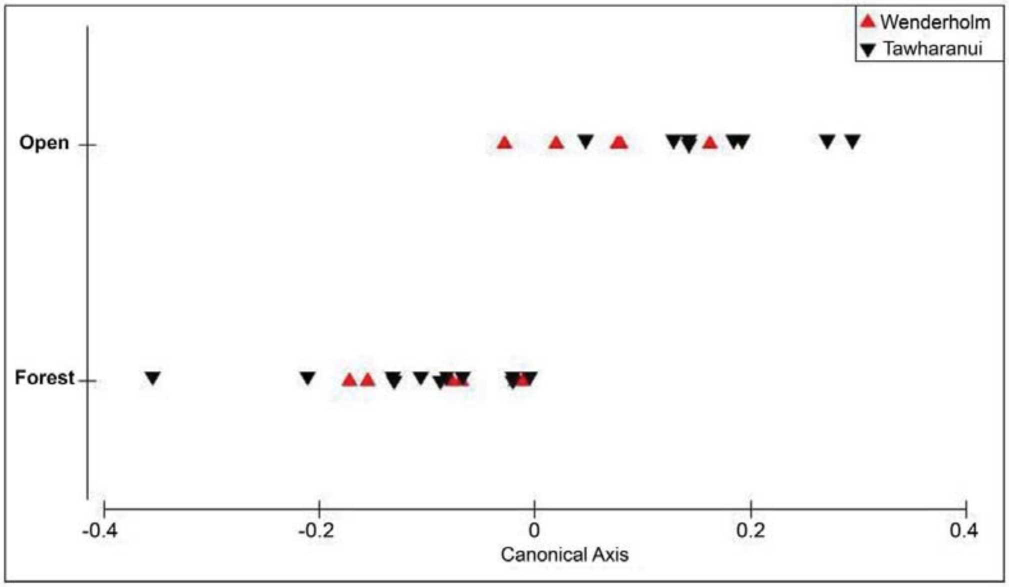


Figure 5.4: A CAP analysis plot showing the forest and open habitats at both Tawharanui, and Wenderholm versus the first canonical axis. The CAP plot chooses an axis that best discriminates the song complexity data and plots the data on that axis. The CAP analysis correctly classified 93.3% of the songs, strongly indicating that a high degree of variation in song complexity between the forest and open habitats existed in both nature reserves.

5.4 Discussion

I have presented strong evidence of tui song complexity variation between two different local habitat types. Consistent with the hypothesis, in forest habitats with a more complex plant community, tui syllable diversity was greater, entropy (spectral complexity) was higher, song duration longer, and trills both longer and of higher proportion. This is the first study to provide evidence for habitat complexity correlating with syllable diversity and entropy in tui.

Longer song duration in forest habitats is consistent with previous research (Handford & Lougheed 1991; Van Dongen & Mulder 2006). Birds occupying territories with denser understoreys sing longer songs (Van Dongen & Mulder 2006). This suggests habitat variation contributes to intraspecific variation in song. Song length increases in more complex habitats are associated with increases in the number of syllables produced (Van Dongen & Mulder 2006).

Trill length increases in closed, dense forest habitats observed in tui is also consistent with previous research (Wiley 1991) and likely relate to acoustic adaptation. Birds produce longer trills to mitigate reverberations in closed habitats that have numerous scattering surfaces such as leaves and branches (Tubaro & Segura 1994). This ‘transmission fidelity’ helps explain individual intraspecific differences in song variables observed in divergent habitat structures (Bertelli & Tubaro 2002; Nemeth et al. 2006).

There are various potential explanations for the observed correlation between song complexity and habitat complexity in tui. I propose the following hypotheses: (i) Intraspecific competition hypothesis: competition between male tui for breeding territory results in more dominant individuals occupying forest habitats with more abundant food resources and more optimal nesting sites. Evidence indicates male songbirds with higher phenotypic quality will have higher song complexity (e.g., Schmidt et al. 2013). Furthermore, song complexity generally increases with both a male's resource holding potential (Grava et al. 2013), social status (Spencer et al. 2004) and competitive ability (Searcy & Nowicki 2005). When individuals vary in competitive ability, a 'despotic distribution' may be evident, where dominant individuals occupy superior habitats and less competitive individuals settle in marginal habitats (Fuller 2012).

(ii) Neighbour effect hypothesis: better quality habitats with more abundant food resources and nesting sites support higher densities of tui. Therefore, individuals will have a greater number of neighbours. Current understanding indicates that high quality habitats, attracting higher degrees of immigration, are positively associated with song repertoire size (Fayet et al. 2014). High immigration could drive higher song complexity in these habitats due to increased intrasexual competition. Furthermore, group size is a fundamental measure of social complexity and can influence song complexity. Specifically, birds in larger social groups may produce higher degrees of song complexity (Freeberg 2006). I did not evaluate tui population data specifically; however, considering this in future could reveal it a contributory factor to differences in song complexity between habitats.

(iii) Developmental advantage hypothesis: forest habitats provide better food resources, therefore better nutrition. This results in a more highly developed telencephalonic nucleus, the HVC, in birds occupying forest habitats. Nutrition during juvenile maturation can affect HVC and song development (Nowicki et al. 2002). Previous studies indicate that in habitats with lower food availability, young songbirds are more likely to suffer nutritional stress. This impairs HVC development (Buchanan et al. 2003; MacDougall-Shackleton 2009; Buchanan 2011), and subsequently reduces song complexity (Schmidt et al. 2014).

The current study does not provide definitive conclusions on which of the above discussed hypotheses apply. Further studies are therefore necessary to fully understand the mechanisms of habitat effects on tui vocal complexity. These should incorporate experimental design testing the effect of (i) varying population density and dispersion; (ii) individual genotypic and phenotypic variation; and (iii) juvenile developmental variation, on tui song complexity. Such research will help clarify causality between habitat type and song complexity variation.

6 Conclusions and future directions

A tui nestling banded at Tawharanui Regional Park (photo: Author).

Overall, the findings in this thesis have provided evidence that several biological and environmental factors are associated with the evolution of a sexually and socially-selected characteristic, song complexity. By presenting comprehensive and novel evidence using observational and experimental approaches, this thesis has provided a further understanding of song complexity evolution in songbirds. Multiple studies have previously proposed that complex songs may be indicators of male quality. Selection pressures generally favour such characteristics, particularly in species with high EPP frequencies because of stronger selection pressures to produce complex songs. The production of more complex songs should theoretically translate into mating and ultimately breeding success (Soma & Garamszegi 2011), and indeed the significant positive association between EPP frequencies and within-song complexity presented within this thesis provides new evidence in support of this hypothesis, across a large number of songbird species. Complex song production is also often associated with the ability to defend territories. Within this thesis, novel empirical evidence was presented that firstly suggests that increases in song complexity characteristics may serve to reduce territorial intrusion rates in a vocally complex species and secondly that the production of complex songs by rival males may be perceived by territorial males as a greater threat perhaps to territory and potential mate cuckoldry. Evidence was presented in this thesis that habitat complexity is associated with two song complexity characteristics (entropy and syllable diversity). Such evidence has never previously been reported, and the findings support the hypothesis that acoustic adaptation to different habitat types can affect intraspecific communication and give rise to variation in song complexity traits.

6.1 Song complexity and mate attraction

I tested whether the evolution of complex broadcast song was associated with sexual selection pressures relating to EPP (Chapter 2). Using data from 78 songbird species, the most comprehensive study in this area to date, EPP frequency was found to be associated significantly and positively with within-song complexity but not between-song complexity. In other words, overall, those species with higher EPP frequencies have more complex songs suggesting that EP mate choice may play a role in the evolution of within-song complexity in broadcast songs. More work is required to fully understand this relationship; however, these results suggest that higher within-song complexity may have evolved to increase mating opportunities in both a social-pair and an EP mating context. The association between EPP frequency and song complexity observed in this study nevertheless is contrary to other similar studies (Garamszegi & Møller 2004; Soma & Garamszegi 2011). These meta-analyses suggested that there were only tenuous associations between EPP and song complexity, however within this thesis, advancing from these previous studies, song complexity data from a larger number of species was extracted and analysed from both online database and field recordings. Furthermore, Soma & Garamszegi (2011) stated that factors such as interspecific variations in breeding systems needed to be considered to fully understand the role song plays in reproductive success. Subsequently, variation in breeding systems was considered and controlled for within this chapter in addition to several other potentially confounding factors such as sexual dichromatism and levels of parental care.

Comparisons of complexity between broadcast songs and interactive songs in tui, a species with very high EPP frequency, also indicated the importance of song complexity in attracting EP mates. In tui, long-range broadcast songs, which are more likely to reach distant audiences including potential EP mates, were found to have higher complexity than interactive songs, which are used in short-range male-male confrontations, courtship and pair-bond maintenance (Titus 1998; Reichard et al. 2011). Complexity in broadcast songs may function to attract EP mates, repel rivals and to minimise social partner cuckoldry.

Future research is required to understand how songbird song complexity varies intraspecifically, and how this will affect the reproductive success of individuals of both sexes. Testing song complexity levels and reproductive success within multiple individuals would expand further our knowledge of song complexity evolution in the context of sexual selection. EPP frequency data in this study was collated from research articles generally based on single study populations. However, the level of EPP can vary markedly between populations of species within the same taxonomic family (Brouwer et al. 2017), with male density and sperm length being found to be key predictors of this variation (Lifjeld et al. 2010; Brouwer et al. 2017). Intraspecific variations in EPP frequency between different populations have also been described (Petrie et al. 1998; Yuta & Koizami 2016). For example, red-winged blackbirds (*Agelaius phoeniceus*) exhibit inter-population variations in EPP frequency ranging between 26-40% (Liu et al. 2015). EPP frequency also may vary between individual males within a population. Further studies are therefore required to investigate whether EPP frequency variation has an effect on song complexity at both the population and individual levels. These will further advance our understanding of the influence of EPP on the evolution of song complexity.

6.2 Song complexity and territoriality

Tui sing more complex songs during the dawn period compared to noon and dusk. Intensified vocal activity at dawn such as a higher number of accented terminal syllables (Morse 1989), and higher song output (Otter et al. 1997; Poesel et al. 2001; Otter & Ratcliffe 2005; Catchpole & Slater 2008; Erne & Amrhein 2008; Sandoval & Mennill 2014; Quispe et al. 2016) has also been found in other songbird species. However, diurnal variations in song complexity remained largely unexplored previously. I subsequently examined the relationship between the song complexity of territorial males and the number of territory intrusions by other males across the diurnal period (Chapter 3). Tui song complexity and intrusion rates were significantly higher at dawn than at solar noon and at dusk. There were also associations between specific song complexity variables and intrusion rate. Specifically, higher entropy and higher syllable rates were inversely correlated with intrusion rate.

The role of song complexity in songbird territoriality was further examined using playback experiments (Chapter 4). Male tui responded more aggressively to complex songs than to simpler songs. For example, they exhibited significantly quicker reactions to complex song stimuli and responded to complex song stimuli with more complex songs. These findings suggest that the more complex songs were perceived as a greater threat by territorial male tui than the simpler songs. In species with high EPP frequency such as tui, complex song is likely to be perceived as a significant threat to territorial males in terms of the potential of losing paternity to rival EP mating (Kokko 1999; Eliassen & Jørgensen 2014; Dowling & Webster 2015). Therefore, aggressive responses of territorial males may mitigate the risk of reduced reproductive fitness.

The findings within these two chapters support the theory that songbird song complexity functions at least partly as a territory defence signal. Higher song complexity observed at dawn than at other periods of the day also heightens evidence for the existing theory that dawn is a critical time of day in songbird communication within a territoriality context. Further experimental efforts using playback approaches should be conducted to test the correlation between song complexity and territorial intrusion. For example, playbacks with complexity variables of differing values should be presented on territories in conjunction with male dummy models, and territorial intrusions on each territory subsequently measured. It is also important to further test the influence of male song complexity on the EPP frequency and the total reproductive success of individual males. Further, it is suggested that dawn (Dalziell & Cockburn 2008), and in particular the dawn chorus, is important for EP mating, since dawn singing may be an honest signal reflecting male quality (Suter et al. 2009). These factors also warrant further research in tui, so as to provide important further insights into the evolution of songbird song complexity.

6.3 Song complexity and habitat type

The results indicated that habitat type was significantly associated with tui song complexity (Chapter 5). While previous studies provided evidence that acoustic adaptation to differing habitat types caused increases in trill proportions and song lengths (Wiley 1991; Van Dongen & Mulder 2006), there was previously no evidence that other song complexity measures such as entropy and syllable diversity varied in differing habitats. Within this thesis, higher entropy and syllable diversity in addition to longer trills and longer songs were found in more complex habitats than in open habitats.

Further studies are however required to test whether variations in song complexity within areas of differing habitat complexity are caused by abiotic factors, e.g. local air turbulence, or biotic factors, i.e. complex habitats provide better and more abundant food resources therefore better developmental nutrition. This would theoretically result in a more highly developed HVC in birds occupying complex forest habitats: the developmental advantage hypothesis; and individuals with higher song complexity will occupy complex habitats through intra-species competition: the intraspecific competition hypothesis. Since there are multiple factors that could potentially explain higher song complexity in more complex habitats, future experimental design is also required to clarify causality between habitat complexity and song complexity variations. These could include testing the effect of varying degrees of male density, presumed competitors, on song complexity. Although challenging in a natural environment, experimental manipulation of male density in behavioural studies has been conducted previously by both the removal of birds from certain habitats (Dhondt 2012), and the addition of individuals to habitats (Sanz 2001), with undisturbed areas acting as control areas. Songs from individuals within habitats of similar complexity levels could then be recorded and complexity measured. Songs from the population should be recorded prior to and after the experimental manipulation. It subsequently could be determined whether there are any variations in song complexity in individuals within these habitats as a consequence of density manipulation. It would be predicted that individuals in the more densely populated habitats would have higher complexity since song complexity has been previously found to be associated with larger population density (Hamao & Ueda 2000), in addition to higher levels of male competition for nesting resources, food, and mates (Craig & Jenkins 1982).

In songbirds, vocalisations are produced by both sexes. Although this thesis focused on male song, recent research suggests that females of 71% of songbird species produce vocalisations (Odom et al. 2014). The role of female song however until recently had been infrequently studied and is still relatively poorly understood (Cain et al. 2015). Brunton et al. (2016) found that song rate and complexity in female bellbirds positively correlated with fledging success of young. Similarly, Cain et al. (2015) found that song rate positively correlated with nesting success. Furthermore, Krieg & Getty (2016) stated that female song in house wrens (*Troglodytes aedon*) may function as a territorial tool against rival females, protecting unguarded eggs within the nest that rivals of both sexes may destroy (Belles-Isles & Picman 1986). However, Odom & Omland (2018) suggested that in a duetting species, the Venezuelan troupial (*Icterus icterus*), female song was found to be less of a threat to rivals than male-female duet song, potentially because it does not signify as great a territorial threat as a duet. These factors suggest that song may not have evolved solely by sexual selection as previously thought but rather may have evolved under other selective pressures such as social or natural selection (Odom et al. 2014). Female song in songbirds therefore should continue to be the subject of experimental research in order to elucidate causality in terms of both reproductive success and territory defence (Hall & Langmore 2017). Future in-depth studies on female song will provide a deeper and more holistic understanding of the evolution and function of song in songbirds.

References

Abdi H. 2007. Z-scores. In: Salkind NJ (ed). Encyclopedia of measurement and statistics. Sage Publications, Thousand Oaks, CA, USA. pp. 1057–1058.

Alatalo RV Lundberg A, Glynn C. 1986. Female pied flycatchers choose territory quality and not male characteristics. Nature 323: 152–153.

Alatalo RV, Glynn C, Lunberg A. 1990. Singing rate and female attraction in the Pied Flycatcher: an experiment. Animal Behaviour 39: 601–603.

Alonso-Alvarez C, Doutrelant C, Sorci G. 2004. Ultraviolet reflectance affects male-male interactions in the blue tit (*Parus caeruleus ultramarinus*). Behavioral Ecology 15: 805–809.

Amrhein V, Kunc HP, Naguib M. 2004. Non-territorial nightingales prospect territories during the dawn chorus. Biology Letters 271: S164–S169.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Anderson MJ, Willis TJ. 2003.Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84: 511–525.

Anderson MJ, Gorley RN. 2008. PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E, Plymouth, UK.

Anderson MJ, Walsh DCI. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecological Monographs 83: 557–574.

Andersson M. 1994. Sexual selection. Princeton University Press, New Jersey, NJ.

Arnold KE, Owens IPF. 2002. Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. Proceedings of the Royal Society of London B: Biological Sciences 269: 1263–1269.

Aubin T. 1981. Etude expérimentale du chant territorial de l'alouette des champs (*Alauda arvensis* L.). Caractéristiques physiques, valeur sémantique et spécificité [PhD] University of Besançon, Nancy 1, Strasbourg.

Avey MT, Quince AF, Sturdy CB. 2008. Seasonal and diurnal patterns of black-capped chickadee (*Poecile atricapillus*) vocal production. Behavioural Processes 77: 149–155.

Badyaev AV, Hill GE, Weckworth BV. 2002. Species divergence in sexually selected traits: Increase in song elaboration is related to decrease in plumage ornamentation in finches. Evolution 56: 412–419.

Baker MC. 1996. Depauperate meme pool of vocal signals in an island population of singing honeyeaters. Animal Behaviour 51: 838–858.

Baker MC, Logue DM. 2003. Population differentiation in a complex bird sound: a comparison of three bioacoustical analysis procedures. Ethology 109: 223–242.

Ballentine B. 2009. The ability to perform physically challenging songs predicts age and size in male swamp sparrows (*Melospiza georgiana*). Animal Behaviour 77: 973–978.

Ballentine B, Badyaev A, Hill GE. 2003. Changes in song complexity correspond to periods of female fertility in blue grosbeaks (*Guiraca caerulea*). Ethology 109: 55–66.

Ballentine, B, Hyman, J, Nowicki S. 2004. Vocal performance influences female response to male bird song: an experimental test. Behavioral Ecology 15: 163–168.

Barger N, Hanson KL, Teffer K, Schenker-Ahmed NM, Semendeferi K. 2014. Evidence for evolutionary specialization in human limbic structures. Frontiers in Human Neuroscience 8: 277.

Bartsch C, Weiss M, Kipper S. 2015. Multiple song features are related to paternal effort in common nightingales. BMC Evolutionary Biology 15: 115.

Beecher MD, Brenowitz EA. 2005. Functional aspects of song learning in songbirds. Trends in Ecology & Evolution 20: 143–149.

Beecher MD, Campbell SE. 2005. The role of unshared songs in singing interactions between neighbouring song sparrows. Animal Behaviour 70: 1297–1304.

Belles-Isles J-C, Picman J. 1986. House wren nest-destroying behavior. Condor 88: 190–193.

Bergquist CAL. 1985. Differences in the diet of the male and female tui (*Prosthemadera novaeseelandiae*: Meliphagidae). New Zealand Journal of Zoology 12: 573–576.

Bergquist CAL, Craig JL. 1988. Competitive assymetries, status and breeding success of tui (Meliphagidae) at an established feeding station. New Zealand Journal of Zoology 15: 369–380.

Bergquist CAL. 1989. Foraging behaviour, social organisation, and use of song by tui in an urban area [PhD] University of Auckland, Auckland, New Zealand.

Bertelli S, Tubaro PL. 2002. Body mass and habitat correlates of song structure in a primitive group of birds. Biological Journal of the Linnean Society 77: 423–30.

Birkhead TR. 1998. Sperm competition in birds. Reviews of Reproduction 3: 123–129.

Bolhuis JJ, Giraldeau L-A. 2005. The study of animal behavior. In: Bolhuis JJ, Giraldeau L-A (eds). The behavior of animals: Mechanisms, function and evolution. Blackwell, NY, USA. pp. 1–9.

Boncoraglio G, Saino N. 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. Functional Ecology 21: 134–142.

Boogert NJ, Giraldeau L-A, Lefebvre L. 2008. Song complexity correlates with learning ability in zebra finch males. Animal Behaviour 76: 1735–1741.

Boogert NJ, Anderson RC, Peters S, Searcy WA, Nowicki S. 2011. Song repertoire size in male song sparrows correlates with detour reaching, but not with other cognitive measures. Animal Behaviour 81: 1209–1216.

Borror DJ. 1981. The songs and singing behavior of the red-eyed vireo. Condor 83: 217–228.

Botero CA, Rossman RJ, Caro LM, Stenzler LM, Lovette IJ, De Kort SR, Vehrencamp SL. 2009. Syllable type consistency is related to age, social status, and reproductive success in the tropical mockingbird. Animal Behaviour 77: 701–706

Botero CA, De Kort SR. 2013. Learned signals and consistency of delivery: a case against receiver manipulation in animal communication. In: Steggman UE (ed). Animal communication theory: information and influence. Cambridge University Press, Cambridge, UK. pp. 281–296.

Boughey MJ, Thompson NS. 1981. Song variety in the brown thrasher (*Toxostoma rufum*). Zeitschrift für Tierpsychologie 56: 47–58.

Bowers EK, Forsman AM, Masters BS, Johnson BGP, Johnson LS, Sakaluk SK, Thompson CF. 2015. Increased extra-pair paternity in broods of aging males and enhanced recruitment of extra-pair young in a migratory bird. Evolution 69: 2533–2541.

Bradbury JW, Vehrencamp SL. 1998. Principles of animal communication. Sinauer, Sunderland, MA, USA.

Briefer E. Aubin, T, Lehongre, K, Rybak F. 2008. How to identify dear enemies: the group signature in the complex song of the skylark *Alauda arvensis*. Journal of Experimental Biology 211: 317–326.

Briefer E, Osiejuk TS, Rybak F, Aubin T. 2010. Are bird song complexity and song sharing shaped by habitat structure? An information theory and statistical approach. Journal of Theoretical Biology 262: 151 –164.

Brouwer L, van de Pol M, Hidalgo Aranzamendi N, Bain G, Baldassarre DT, Brooker LC, Brooker MG, Colombelli-Négrel D, Enbody E, Gielow K, Hall ML, Johnson AE, Karubian J, Kingma SA, Kleindorfer S, Louter M, Mulder RA, Peters A, Pruett-Jones S, Tarvin KA, Thrasher DJ, Varian-Ramos CW, Webster MS, Cockburn A. 2017. Multiple hypotheses explain variation in extra-pair paternity at different levels in a single bird family. Molecular Ecology doi: 10.1111/mec.14385.

Brown TJ, Handford P. 2003. Why birds sing at dawn: the role of consistent song transmission. Ibis 145: 120–129.

Brumm H. 2004. The impact of environmental noise on song amplitude in a territorial bird. Journal of Animal Ecology 73: 434–400.

Brumm H, Todt D. 2004. Male-male vocal interactions and the adjustment of song amplitude in a territorial bird. Animal Behaviour 67: 281–286.

Brumm H, Slater PJB. 2006. Ambient noise, motor fatigue and serial redundancy in chaffinch song. Behavioral Ecology and Sociobiology 60: 475–81.

Brumm H, Lachlan RF, Riebel K, Slater PJB 2009. On the function of song type repertoires: testing the ‘antiexhaustion hypothesis’ in chaffinches. Animal Behaviour 77: 37–42.

Brumm H, Ritschard M. 2011. Song amplitude affects territorial aggression of male receivers in chaffinches. Behavioral Ecology 22: 310–316.

Brunner P, Pasinelli G. 2010. Variation in singing style use in the reed bunting *Emberiza schoeniclus*: influencing factors and possible functions. Journal of Avian Biology 41: 388–397.

Brunton DH, Li X. 2006. The song structure and seasonal patterns of vocal behavior of male and female bellbirds (*Anthornis melanura*). Journal of Ethology 24: 17–25.

Brunton DH, Roper MM, Harmer AMT. 2016. Female song rate and structure predict reproductive success in a socially monogamous bird. Frontiers in Ecology and Evolution 4: 1–11.

Buchanan KL, Catchpole CK. 1997. Female choice in the sedge warbler *Acrocephalus schoenobaenu*s multiple cues from song and territory quality. Proceedings of the Royal Society of London B: Biological Sciences 264: 521–526.

Buchanan KL, Catchpole CK, Lewis JW, Lodge A. 1999. Song as an indicator of parasitism in the sedge warbler. Animal Behaviour 57: 307–314.

Buchanan KL, Catchpole CK. 2000. Song as an indicator of male parental effort in the sedge warbler. Proceedings of the Royal Society of London B: Biological Sciences 267: 321–326.

Buchanan KL, Leitner S, Spencer KA, Goldsmith AR, Catchpole CK. 2003. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. Proceedings of the Royal Society of London B: Biological Sciences 271: 2381–2386.

Buchanan KL, Leitner S, Spencer KA, Goldsmith AR, Catchpole CK. 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. Philosophical Transactions of the Royal Society B 271: 2381–2386.

Buchanan KL. 2011. The developmental stress hypothesis: a special case of the evolution of condition-dependent sexual traits. Behavioral Ecology 22: 12–13.

Burkett ZD, Day NF, Peñagarikano O, Geschwind DH, White SA. 2015. VoICE: A semi-automated pipeline for standardizing vocal analysis across models. Scientific Reports 5: 10237.

Burnham CA, Anderson DR. 2002. Model selection and multimodel inference: a practical information-theoretic approach (4th ed.). Springer, New York, NY, USA.

Burns KC, Lake B. 2009. Fruit-frugivore interactions in two southern hemisphere forests: allometry, phylogeny and body size. Oikos 118: 1901–1907.

Butler SR, Fernández-Juricic E. 2014. European starlings recognize the location of robotic conspecific attention. Biology Letters 10: 20140665.

Byers BE. 2007. Extrapair paternity in chestnut-sided warblers is correlated with consistent vocal performance. Behavioral Ecology, 18: 130–136.

Byers BE, Kroodsma DE. 2009. Female mate choice and songbird song repertoires. Animal Behaviour 77: 13–22.

Cain KE, Cockburn A, Langmore NE. 2015. Female song rates in response to simulated intruder are positively related to reproductive success. Frontiers in Ecology and Evolution 3: 119.

Canal D, Jovani R, Potti J. 2012. Male decisions or female accessibility? Spatiotemporal patterns of extra pair paternity in a songbird. Behavioral Ecology 23: 1146–1153.

Catchpole CK. 1980. Sexual selection and the evolution of complex songs among European warblers of the genus Acrocephalus. Behaviour 74: 149–166.

Catchpole CK. 1980. Sexual selection and the evolution of complex songs among European warblers of the genus Acrocephalus. Behaviour 74: 149–166.

Catchpole CK. 1983. Variation in the song of the great reed warbler *Acrocephalus arundinaceus* in relation to mate attraction and territorial defence. Animal Behaviour 31: 217–1225.

Catchpole CK, Dittami J, Leisler B. 1984. Differential responses to male song repertoires in female songbirds implanted with oestradiol. Nature 312: 563–564.

Catchpole CK. 1986. Song repertoires and reproductive success in the great reed warbler *Acrocephalus arundinaceus*. Behavioral Ecology and Sociobiology 19: 439–445.

Catchpole CK, Leisler B, Dittami J. 1986. Sexual differences in the responses of captive great reed warblers *Acrocephalus arundinaceus*, to variation in song structure and size. Ethology 73: 69–77.

Catchpole CK. 1992. Integrating playback: a wider context. In: McGregor PK (ed). Playback and studies of animal communication. Springer Science and Business Media, Berlin, Germany. pp. 35–46.

Catchpole CK, Slater PJB. 2008. Bird song: Biological themes and variations, 2nd ed. Cambridge University Press, Cambridge, UK.

Chaine AS, Montgomerie R, Lyon BE. 2015. Sexual conflict arising from extrapair matings in birds. Cold Spring Harbor perspectives in biology 7: a017590.10.1101.

Charif RA, Waack AM, Strickman LM. 2010. Raven Pro 1.4 user’s manual. Cornell Lab of Ornithology, Ithaca, NY, USA.

Clarke KR, Warwick RM. 1994. Similarity-based testing for community pattern: the 2-way layout with no replication. Marine Biology 118: 167–176.Clarke KR, Gorley RN. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, UK.

Cody ML, Stabler E, Castellanos HMS, Taylor CE. 2016. Structure, syntax and "small-world" organization in the complex songs of California thrashers (*Toxostoma redivivum*). Bioacoustics 25: 1–14.

Cottam G, Curtis JJ. 1956. The use of distance measures in phytosociological sampling. Ecology 37: 451–460.

Craig JL, Stewart AM, Douglas ME. 1981. The foraging of New Zealand honeyeaters. New Zealand Journal of Zoology 8: 87–91.

Craig JL, Jenkins PF 1982. The evolution of complexity in broadcast song of passerines. Journal of Theoretical Biology 95: 415–422.

Craig JL. 1984. Wing noises, wing slots, and aggression in New Zealand honeyeaters (aves, meliphagidae). New Zealand Journal of Zoology 11: 195–199.

Cramer ERA. 2013. Physically challenging song traits, male quality, and reproductive success in house wrens. PLOS ONE 8(3): e59208.

Croston R, Branch CL, Kozlovsky DY, Dukas R, Pravosudov VV. 2015. Heritability and the evolution of cognitive traits. Behavioral Ecology 26: 1447–1459.

Dabelsteen T, McGregor PK, Lampe HM, Langmore NE, Holland J. 1998. Quiet song in song birds: an overlooked phenomenon. Bioacoustics 9: 89–105.

Dabelsteen T, Mathevon N. 2002. Why do songbirds sing intensively at dawn? A test of the acoustic transmission hypothesis. Acta Ethologica 4: 65–72.

Dalziell AH, Cockburn A. 2008. Dawn song in superb fairy-wrens: a bird that seeks extra-pair copulations during the dawn chorus. Animal Behaviour 75: 489–500.

Dalziell AH, Magrath RD. 2012. Fooling the experts: accurate vocal mimicry in the song of the superb lyrebird, *Menura novaehollandiae*. Animal Behaviour 83: 1401e14101402.

Dalziell AH, Peters RA, Cockburn A, Dorland AD, Maisey AC, Magrath RD. 2013. Dance choreography is coordinated with song repertoire in a complex avian display. Current Biology 23: 1132–1135.

De Groof G, Verhoye M, Poirier C, Leemans A, Eens M, Darras VM, Van der Linden A. 2009. Structural changes between seasons in the songbird auditory forebrain. Journal of Neuroscience 29: 13557–13565.

De Kort SR, Eldermire ERB, Cramer ERA, Vehrencamp SL. 2009. The deterrent effect of bird song in territory defense. Behavioral Ecology 20: 200–206.

Derégnaucourt S, Saar S, Gahr M. 2012. Melatonin affects the temporal pattern of vocal signatures in birds. Journal of pineal research 53: 245–258.

DeVoogd TJ, Krebs JR, Healy SD, Purvis A. 1993. Relations between song repertoire size and volume of brain nuclei related to song: comparative evolutionary analyses amongst oscine birds. Proceedings of the Royal Society of London B: Biological Sciences 254: 75–82.

Dhondt AA. 2012. Interspecific competition in birds. Oxford University Press, NY, USA.Doutrelant C, Blondel J, Perret P, Lambrechts MM. 2000. Blue tit song repertoire size, male quality and interspecific competition. Journal of Avian Biology 31: 360–366.

Dowling, J, Webster MS. 2015. An experimental test of duet function in a fairy-wren (*Malurus*) with moderate cuckoldry rates. Behavioral Ecology 27: 228–236.

Drăgănoiu TI, Nagle L, Kreutzer M. 2002. Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. Proceedings of the Royal Society of London B: Biological Sciences 269: 2525–2531.

DuBois AL, Nowicki S, Searcy WA. 2009. Swamp sparrows modulate vocal performance in an aggressive context. Biology Letters 5: 163–165.

Eens M, Pinxten R, Verheyen RF. 1991. Male song as a cue for mate choice in the European starling. Behaviour 116, 210–238.

Eliassen S, Jørgensen C. 2014. Extra-pair mating and evolution of cooperative neighbourhoods. PLOS ONE. 9: e99878.

Erne N, Amrhein V. 2013. Long-term influence of simulated territorial intrusions on dawn and dusk singing in the winter wren: spring versus autumn. Journal of Ornithology 149: 479–486.

Ewenson EL, Zann RA, Flannery GR. 2001. Body condition and immune response in wild zebra finches: effects of capture, confinement and captive-rearing. Naturwissenschaften 88 391–394.

Falls JB. 1988. Does song deter intrusion in white-throated Sparrows (*Zonotrichia albicollis*)? Canadian Journal of Zoology 66: 206–211.

Falls JB. 1992. Playback: a historical perspective. In: McGregor PK (ed). Playback and studies of animal communication. Plenum Press, New York, NY, USA. pp. 11–33.

Fayet AL, Tobias JA, Hintzen RE, Seddon N. 2014. Immigration and dispersal are key determinants of cultural diversity in a songbird population. Behavioral Ecology 25: 744–753.

Feßl B, Hoi H. 2000. Song complexity and song structure in the moustached warbler *Acrocephalus melanopogon*. Journal of Avian Biology 31: 144–150.

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425: 714–717.

Forstmeier W, Kempenaers B, Meyer A, Leisler B. 2002. A novel song parameter correlates with extra-pair paternity and reflects male longevity. Proceedings of the Royal Society of London B: Biological Sciences 269: 1479–1485.

Forstmeier W, Nakagawa S, Griffith SC, Kempenaers B. 2014. Female extra-pair mating: adaptation or genetic constraint? Trends in Ecology & Evolution 29: 456–464.

Freeberg TM. 2006. Social complexity can drive vocal complexity: group size influences vocal information in Carolina chickadees. Psychological Science 17: 557–561.

Fuller RJ. 2012. Habitat quality and habitat occupancy by birds in variable environments. In: Fuller RJ (ed). Bird and habitat: relationships in changing landscapes. Cambridge University Press, New York, NY, USA. pp. 93–124.

Funghi C, Cardoso GC, Mota PG. 2014. Increased syllable rate during aggressive singing in a bird with complex and fast song. Journal of Avian Biology 46: 283–288.

Garamszegi LZ, Møller AP, Erritzøe J. 2003. The evolution of immune defense and song complexity in birds. Evolution 57: 905–912.

Garamszegi LZ, Møller AP. 2004. Extrapair paternity and the evolution of bird song. Behavioral Ecology 15: 508–519.

Garamszegi LZ, Moreno J, Møller AP. 2006. Avian song complexity is associated with high field metabolic rate. Evolutionary Ecology Research 8: 75–90.

Garamszegi LZ, Zsebők S, Török J. 2012. The relationship between syllable repertoire similarity and pairing success in a passerine bird species with complex song. Journal of Theoretical Biology 295: 68–76.

Gardner TJ, Naef F, Nottebohm F. 2005. Freedom and rules: the acquisition and reprogramming of a bird's learned song. Science 308: 1046–1049.

Geberzahn N, Aubin T. 2014. Assessing vocal performance in complex birdsong: a novel approach. BMC Biology 12: 58.

Gerlach NM, McGlothlin JW, Parker, PG, Ketterson ED. 2012. Reinterpreting Bateman gradients: multiple mating and selection in both sexes of a songbird species. Behavioral Ecology 23: 1078–1088.

Gil D, Gahr M. 2002. The honesty of bird song: multiple constraints for multiple traits. Trends in Ecology & Evolution 17: 133–141.

Gil D, Slater PJB. 2000. Multiple song repertoire characteristics in the willow warbler (*Phylloscopus trochilus*): correlations with female choice and offspring viability. Behavioral Ecology and Sociobiology 47: 319–326.

Gil D, Slater PJB. 2000. Song organisation and singing patterns of the willow warbler *Phylloscopus trochilus*. Behaviour 137: 759–782.

Gil D, Slater PJB, Graves JA. 2007. Extra-pair paternity and song characteristics in the willow warbler *Phylloscopus trochilus*. Journal of Avian Biology 38: 291–297.

Glaze CM, Troyer TW. 2006. Temporal structure in zebra finch song: implications for motor coding. Journal of Neuroscience 26: 991–1005.

Gotelli NJ, Colwell RK. 2010. Estimating species richness. In: Magurran AE, McGill BJ (eds). Biological diversity: frontiers in measurement and assessment. Oxford University Press, Oxford, UK. pp. 39–54.

Gould JL. 2004. Learning instincts. In: Pashler H, Gallistel R (eds). Stevens' Handbook of Experimental Psychology, Volume 3, Learning, Motivation, and Emotion, 3rd ed. Wiley, Hoboken, NJ, USA. pp. 239–258.

Grava T, Grava A, Otter KA. 2013. Habitat-induced changes in song consistency affect perception of social status in male chickadees. Behavioral Ecology and Sociobiology 67: 1699–1707.

Gray EM. 1997. Female red-winged Blackbirds accrue material benefits from copulating with extra-pair males. Animal Behaviour 53: 625–639.

Greig-Smith PW. 1982. Seasonal patterns of song production by male stonechats, Saxicola torquata. Ornis Scandinavica 13: 225–231.

Gross K, Pasinelli G, Kunc HP. 2010. Behavioral plasticity allows short-term adjustment to a novel environment. American Naturalist 176: 454-464.

Grunst ML, Rotenberry JT, Grunst AS. 2016 Elevating perceived predation risk modifies the relationship between parental effort and song complexity in the song sparrow *Melospiza melodia*. Journal of Avian Biology 47: 57–68.

Habib L, Bayne EM, Boutin S. 2007. Chronic industrial noise affects pairing success and age structure of ovenbirds *Seiurus aurocapilla*. Journal of Applied Ecology 44: 176–184.

Hall ML, Kingma SA, Peters A. 2013. Male songbird indicates body size with low-pitched advertising songs. PLOS ONE 8(2): e56717.

Hamao S, Ueda K 2000. Simplified song in an island population of the bush warbler *Cettia diphone*. Journal of Ethology 18: 53–57.

Hall ML, Langmore NE. 2017. Editorial: Fitness costs and benefits of female song. Frontiers in Ecology and Evolution 5: 48.

Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: A role for parasites? Science 218: 384–387.

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electron 4:9.

Hamao S, Ueda K. 2000. Simplified song in an island population of the bush warbler *Cettia diphone.* Journal of Ethology 18: 53–57.

Hamao S, Sugita N, Nishiumi I. 2013. Geographical variation in mitochondrial DNA and vocalizations in two resident bird species in the Ryukyu Archipelago, Japan. Bulletin of the National Museum of Nature and Science A 39: 51–62.

Handford P, Nottebohm F. 1976. Allozymic and morphological variation in population samples of rufous- collared sparrow, *Zonotrichia capensis*, in relation to vocal dialects. Evolution 30: 802–817.

Handford P, Lougheed SC. 1991. Variation in duration and frequency characters in the song of the rufous-collared sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects and body size. Condor 93: 644–658.

Harper DGC. 1988. *Erithacus rubecula* robin - Social pattern and behaviour; Voice. In: Cramp S (ed). Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic Volume 5. Oxford University Press, Oxford, UK and New York, NY, USA. pp. 603–612.

Harper SL, Vallender R, Robertson RJ. 2010. Male song variation and female mate choice in the golden-winged warbler. Condor 112: 105–114.

Hart PJ, Sebastián González E, Tanimoto A, Thompson A, Speetjens T, Hopkins M, Atencio-Picado M. 2018. Birdsong characteristics are related to fragment size in a neotropical forest. Animal Behaviour 137: 45–52.

Hasselquist D, Bensch S, Vonschantz T. 1995. Low frequency of extrapair paternity in the polygynous great reed warbler, *Acrocephalus arundinaceus*. Behavioral Ecology 6: 27–38.

Hasselquist, D., Bensch, S., Von Schantz, T. 1996. Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. Nature 381, 229–232.

Hasselquist D. 1998. Polygyny in great reed warblers: a long-term study of factors contributing to male fitness. Ecology 79: 2376–2390.

Hasselquist D, Sherman PW. 2001. Social mating systems and extrapair fertilizations in passerine birds. Behavioral Ecology 12: 457–466. Hiebert SM, Stoddard PK, Arcese P. 1989.

Hennin HL, Barker NKS, Bradley DW, Mennill DJ. 2009. Bachelor and paired male rufous-and-white wrens use different singing strategies. Behavioral Ecology and Sociobiology 64: 151–159.

Hesler N, Mundry R, Dabelsteen T. 2011. Does song repertoire size in common blackbirds play a role in an intra-sexual context? Journal of Ornithology 152: 591–601.

Hiebert SM, Stoddard PK, Arcese P. 1989. Repertoire size, territory acquisition, and reproductive success in the song sparrow. Animal Behaviour 37: 266–273.

Hilje B, Stack S, Sánchez-Azofeifa A. 2017. Lianas abundance is positively related with the avian acoustic community in tropical dry forests. Forests 8: 311.

Hill CE, Akcay C, Campbell SE, Beecher MD. 2011. Extrapair paternity, song, and genetic quality in song sparrows. Behavioral Ecology 22: 73–81.

Hill SD. 2011. The vocalisation of tui (*Prosthemadera novaeseelandiae*) [MSc] Massey University, Auckland, New Zealand.

Hill SD, Ji W, Parker KA, Amiot C, Wells SJ. 2013. A comparison of vocalisations between mainland tui (*Prosthemadera novaeseelandiae novaeseelandiae*) and Chatham Island tui (*P. n. chathamensis*). New Zealand Journal of Ecology 37: 214–223.

Hill SD, Ji W. 2013. Microgeographic variation in song phrases of tui (*Prosthemadera novaeseelandiae*). Notornis 60: 262–264.

Hill SD, Ji, W. 2014. Categorisation of common syllable types in the complex vocalisations of tui (*Prosthemadera novaeseelandiae*). Notornis 61: 54–56.

Hill SD, Amiot C, Ludbrook MR, Ji W. 2015. Seasonal variation in the song structure of tui (*Prosthemadera novaeseelandiae*). New Zealand Journal of Ecology 39: 110–115.

Hill SD, Pawley MDM, Ji W. 2017. Local habitat complexity correlates with song complexity in a vocally elaborate honeyeater. Austral Ecology 42: 590–596.

Hjernquist MB, Thuman Hjernquist KA, Forsmana JT, Gustafsson L. 2008. Sex allocation in response to local resource competition over breeding territories. Behavioral Ecology 20: 335–339.

Huang H, Rabosky DL. 2015. Sex-linked genomic variation and its relationship to avian plumage dichromatism and sexual selection. BMC Evolutionary Biology 15: 199.

Hultsch H, Todt D. 2004. Learning to sing. In: Marler PR, Slabbekoorn H (eds). Nature's music: the science of birdsong. Elsevier, San Diego, CA, USA. pp. 80–107.

Illes AE, Hall ML, Vehrencamp SL. 2006. Vocal performance influences male receiver response in the banded wren. Proceedings of the Royal Society of London B: Biological Sciences 273: 1907–1912.

Jaška P, Linhart P, Fuchs R. 2015. Neighbour recognition in two sister songbird species with a simple and complex repertoire – a playback study. Journal of Avian Biology 45: 151–158.

Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. 2012. The global diversity of birds in space and time. Nature 491: 444–448.

Jetz W, Thomas GH, Joy JB, Hartmann K, Redding D, Mooers AO. 2014. Distribution and conservation of global evolutionary distinctness in birds. Current Biology 24: 1–12.

Jurisevic MA, Sanderson KJ. 1994. The vocal repertoires of six honeyeater (Meliphagidae) species from Adelaide, South Australia. Emu 94: 141–148.

Kacelnik A, Krebs JR. 1982. The dawn chorus in the great tit (*Parus major*): proximate and ultimate causes. Behaviour 83: 287–309.

Kagawa H, Soma M. 2013. Song performance and elaboration as potential indicators of male quality in Java sparrows. Behavioural Processes 99: 138–144.

Kaluthota CD. 2016. Sexual selection, breeding behaviour and song communication in house wrens (*Troglodytes aedon*) [PhD] University of Lethbridge, Canada.

Kelly D, Ladley JJ, Robertson AW, Anderson SH, Wotton DM, Wiser SK. 2010. Mutualisms with the wreckage of an avifauna: the status of bird pollination and fruit dispersal in New Zealand. New Zealand Journal of Ecology 34: 66–85.

Kempenaers B, Verheyen GR, Van den Broeck M, Burke T, Van Broeckhoven C, Dhondt A. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. Nature 357: 494–496.

Kiefer S, Spiess A, Kipper S, Mundry R, Sommer C., Hultsch H, Todt, D 2006. First year common nightingales (*Luscinia megarhynchos*) have smaller repertoire sizes than older males. Ethology 112: 1217–1224.

Kiefer S, Sommer C, Scharff C, Kipper S, Mundry R. 2009. Tuning towards tomorrow? Common nightingales *Luscinia megarhynchos* change and increase their song repertoires from the first to the second breeding season. Journal of Avian Biology 40: 231–236.

Kipper S, Mundry R, Sommer C, Hultsch H, Todt D. 2006. Song repertoire size is correlated with body measures and arrival date in common nightingales*, Luscinia megarhynchos*. Animal Behaviour 71: 211–217

Kipper S, Kiefer S, Bartsch C, Weiss M. 2015. Female calling? Song responses to conspecific call playbacks in nightingales, *Luscinia megarhynchos*. Animal Behaviour 100: 60–66.

Kokko H. 1999. Cuckoldry and the stability of biparental care. Ecology Letters 2: 247–255.

Kokko H, Mappes J. 2013. Multiple mating by females is a natural outcome of a null model of mate encounters. Entomologia Experimentalis et Applicata 146: 26–37.

Krieg CA, Getty T. 2016. Not just for males: females use song against male and female rivals in a temperate zone songbird. Animal Behaviour 113: 39–47.

Kroodsma DE. 1977. Correlates of song organisation among North American wrens. The American Naturalist 111: 995–1008.

Kroodsma DE. 1979. Vocal dueling among male marsh wrens: evidence for ritualized expressions of dominance/subordinance. The Auk 96: 506–515.

Kroodsma DE. 1980. Winter wren singing behavior: a pinnacle of song complexity. Condor 82: 356–365.

Kroodsma DE. 1981. Geographical variation and functions of song types in warblers (Parulidae). The Auk 98: 743–751.

Kroodsma, DE. 1990. Using appropriate experimental designs for intended hypotheses in 'song' playbacks, with examples for testing effects of song repertoire sizes. Animal Behaviour 40: 1138–1150.

Kroodsma DE, Byers BE. 1991. The function(s) of bird song. American Zoologist 31: 318–328.

Kroodsma DE. 2005. The singing life of birds: the art and science of listening to birdsong. Houghton Mifflin Company, Boston, MA, USA.

Kubli SP, MacDougall-Shackleton EA. 2014. Developmental timing of signals affects information content: song complexity but not song consistency reflects innate immune strategy in male song sparrows. The American Naturalist 183: 660–670.

Kunc HP, Amrhein V, Naguib M. 2005. Seasonal variation in dawn song characteristics in the common nightingale. Animal Behaviour 70: 1265–1271.

Lailvaux SP, Irschick DJ. 2006. A functional perspective on sexual selection: insights and future prospects. Animal Behaviour 72: 263–273.

Laiolo P, Tella JL, Carrete M, Serrano D, López G. 2004. Distress calls may honestly signal bird quality to predators. Proceedings of the Royal Society of London B: Biological Sciences 271: S513-S515.

Laiolo P, Vögeli M, Serrano D, Tella JL. 2008. Song diversity predicts the viability of fragmented bird populations. PLOS ONE 3(3): e1822.

Lampe HM, Espmark YO. 1994. Song structure reflects male quality in pied flycatchers, *Ficedula hypoleuca*. Animal Behaviour 47: 869–876.

Lattin C, Ritchison G. 2009. Intra- and intersexual functions of singing by male blue grosbeaks: the role of within-song variation. The Wilson Journal of Ornithology 121: 714–721.

Leedale AE, Collins SA, De Kort SR. 2015. Blackcaps (*Sylvia atricapilla*) increase the whistle part of their song in response to simulated territorial intrusion. Ethology 121: 403–409.

Leisler B, Catchpole CK. 1992. The evolution of polygamy in European reed warblers of the genus Acrocephalus: a comparative approach. Ethology, Ecology & Evolution 4: 225–243.

Leisler B, Beier J, Staudter H, Wink M. 2000. Variation in extra-pair paternity in the polygynous great reed warbler (*Acrocephalus arundinaceus*). Journal of Ornithology 141: 77– 84.

Leitão A, ten Cate C, Riebel K. 2006. Within-song complexity in a songbird is meaningful to both male and female receivers. Animal Behaviour 71: 1289–1296.

Leitner S, Voigt C, Gahr M. 2001. Seasonal changes in the song pattern of the non-domesticated island canary (*Serinus canaria*), a field study. Behaviour 138: 885–904.

Leniowski K, Wegrzyn E. 2013. Organization, variation in time, and impacting factors in the song strophe repertoire in the Great reed warbler (*Acrocephalus arundinaceus*). Ornis Fennica 90: 129–141.

Lifjeld JT, Robertson RJ. 1992. Female control of extra-pair fertilization in tree swallows. Behavioral Ecology and Sociobiology 31: 89–96.

Linhart P, Jaska P, Petrusková T, Petrusek Adam, Fuchs R. 2013. Being angry, singing fast? Signalling of aggressive motivation by syllable rate in a songbird with slow song. Behavioural Processes 100: 139–145.

Liu WC. 2004. The effect of neighbours and females on dawn and daytime singing behaviours by male chipping sparrows. Animal Behaviour 68: 39–44.

Liu IA, Johndrow JE, Lüpold S, Yasukawa K, Westneat DF, Nowicki S. 2015. Genetic diversity does not explain variation in extra-pair paternity in multiple populations of a songbird. Journal of Evolutionary Biology 28: 1156–1169.

Ludbrook MR. 2015. Effects of urban noise on vocalisations of tūī (*Prosthemadera novaeseelandiae*) [MSc] Massey University, Auckland, New Zealand.

Lynch A. 1996. The population memetics of birdsong. In: Kroodsma DE, Miller EH (eds). Ecology and evolution of acoustic communication in birds. Cornell University Press, Ithaca, NY, USA. pp. 181-197.

MacDougall-Shackleton SA. 2009. The importance of development: what songbirds can teach us. Canadian Journal of Experimental Psychology 63: 74–79.

MacDougall-Shackleton EA, Stewart KA, Potvin DA, Tennenhouse E. 2009. The rich get richer: song complexity predicts song element sharing and song output in song sparrows *Melospiza melodia*. Animal Behaviour 78: 141–146.

Mace R. 1987. The dawn chorus in the great tit *Parus major* is directly related to female fertility. Nature 330: 745–746.

Markowitz JE, Ivie E, Kligler L, Gardner TJ. 2013. Long-range order in canary song. PLOS Computational Biology 9(5): e1003052.

Marler P, Tamura M. 1962. Song 'dialects' in three populations of white-crowned sparrows. Condor 64: 368–377.

Marshall RC, Buchanan KL, Catchpole CK. 2003. Sexual selection and individual genetic diversity in a songbird. Proceedings of the Royal Society of London B: Biological Sciences 270: S248–250

Marshall RC, Buchanan KL, Catchpole CK. 2007. Song and female choice for extrapair copulations in the sedge warbler, *Acrocephalus schoenobaenus*. Animal Behaviour 73: 629–635.

Mason NA, Burns KJ, Tobias JA, Claramunt S, Seddon N, Derryberry EP. 2017. Song evolution, speciation, and vocal learning in passerine birds. Evolution 71: 786–796.

McGregor PK, Horn AG. 1992. Strophe length and response to playback in great tits. Animal Behaviour 43: 667–676.

Miller EH. 1982. Character and variance shift in acoustic signals. In: Kroodsma DE, Miller EH (eds.). Acoustic communication in birds, Vol. 1. Academic Press, NY, NY, USA. pp. 253–295.

Moiron M., González-Lagos C, Slabbekoorn H, Sol D. 2015. Singing in the city: high song frequencies are no guarantee for urban success in birds. Behavioral Ecology 26: 843–850.

Møller AP. 1988. Spatial and temporal distribution of song in the yellowhammer *Emberiza cintrinella*. Ethology 78: 321–331.

Møller AP. 1991. Density-dependent extra-pair copulations in the swallow *Hirundo rustica*. Ethology 87: 316–329.

Møller AP. 2000. Male parental care, female reproductive success, and extrapair paternity. Behavioral Ecology 11: 161–168.

Monbureau M, Barker JM, Leboucher G, Balthazart J. 2015. Male song quality modulates c-Fos expression in the auditory forebrain of the female canary. Physiology & Behavior 147: 7–15.

Moore JM, Székely T, Büki J, DeVoogd TJ. 2011. Motor pathway convergence predicts syllable repertoire size in oscine birds. Proceedings of the National Academy of Sciences of the United States of America 108: 16440–16445.

Moorman S, Bolhuis JJ. 2013. Behavioral similarities between birdsong and spoken language. In: Bolhuis JJ, Everaert M (eds). Birdsong, speech and language: exploring the evolution of mind and brain. MIT Press, Cambridge, MA, USA. pp. 111–123.

Morse DH. 1989. Song patterns of warblers at dawn and dusk. Wilson Bulletin 101: 26–35.

Morton ES. 1975. Ecological sources of selection on avian sounds. The American Naturalist 109: 17-34.

Motes-Rodrigo A., Labra A., Lampe HM. 2017. Breeding experience and not age modulates the song development of pied flycatchers (*Ficedula hypoleuca*). Ethology 123*:* 197–204.

Moulton LL, Linz GM, Bleier WJ. 2013. Responses of territorial and floater male red-winged Blackbirds to models of receptive females. Journal of Field Ornithology 84: 160–170.

Mountjoy J, Lemon RE. 1991. Song as an attractant for male and female European starlings, and the influence of song complexity on their response. Behavioral Ecology and Sociobiology 28: 97–100.

Mountjoy DJ, Lemon RE. 1996. Female choice for complex song in the European starling: a field experiment. Behavioral Ecology and Sociobiology 38: 65–71.

Naugler CT, Smith PC. 1991. Song similarity in an isolated population of fox sparrows (*Passerella iliaca*). Condor 93: 1001–1003.

Naugler CT 1992. Effects of the acoustic environment on song structure and song recognition in the American tree sparrow (*Spizella arborea*). [MSc] Queen's University, New York, USA.

Naugler CT, Ratcliffe L. 1994. Character release in bird song: a test of the acoustic competition hypothesis using American tree sparrows *Spizella arborea*. Journal of Avian Biology 25: 142–148.

Nelson DA, Croner LJ. 1991. Song categories and their functions in the field sparrow (*Spizella pusilla*). The Auk 108: 42–52.

Nelson DA, Marler P. 1994. Selection-based learning in bird song development. Proceedings of the National Academy of Sciences of the United States of America 91: 10498–10501.

Nemeth E, Dabelsteen T, Pedersen SB, Winkler H. 2006. Rainforests as concert halls for birds: are reverberations improving sound transmission of long song elements. Journal of the Acoustic Society of America 119: 620–626.

Neto JM, Hansson B, Hasselquist D. 2010. Low frequency of extra-pair paternity in Savi's Warblers (Locustella luscinioides). Behaviour 147: 1413 –1429.

Nolan V. Jr, Ketterson ED, Cristol DA, Rogers CM, Clotfelter ED, Titus R., Schoech SJ, Snajdr E. 2002. Dark-eyed Junco (*Junco hyemalis*). In: Powell A, Gill F (eds). The Birds of North America 176. Birds of North America, Philadelphia, PA, USA. pp. 1-44

Nordby JC, Campbell SE, Beecher MD. 1999. Ecological correlates of song learning in song sparrows. Behavioral Ecology 10: 287–297.

Nottebohm F. 1981. A brain for all seasons: cyclic anatomical changes in song control nuclei of the canary brain. Science 214: 1368–1370.

Nowicki S, Peters S, Podos J. 1998. Song learning, early nutrition and sexual selection in songbirds. American Zoologist 38: 179–190.

Nowicki S, Podos J. 1993. Complexity, coupling and contingency in birdsong. In: Bateson PPG, Klopfer P, Thompson N (eds). Perspectives in Ethology. Plenum Press, NY, USA. pp. 159–186.

Nowicki S, Peters S, Podos J, 1998. Song learning, early nutrition and sexual selection in songbirds. American Zoologist 38: 179–190.

Nowicki S, Hasselquist D, Bensch S, Peters S. 2000. Nestling growth and song repertoire size in great reed warblers: evidence for song learning as an indicator mechanism in mate choice. Philosophical Transactions of the Royal Society B 267: 2419–2424.

Nowicki S, Searcy WA, Peters S. 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the “nutritional stress hypothesis.” Journal of Comparative Physiology A 188: 1003–1014.

Nowicki S, Searcy WA. 2004. Song function and the evolution of female preferences. Annals of the New York Academy of Sciences 1016: 704–723.

Odom KJ, Hall ML, Riebel K, Omland KE, Langmore NE. 2014. Female song is widespread and ancestral in songbirds. Nature Communications 5: 3379.

Odom KJ, Omland KE. 2018. Females and males respond more strongly to duets than to female solos: comparing the function of duet and solo singing in a tropical songbird (*Icterus icterus*). Behaviour DOI: 10.1163/1568539X-00003473.

Okanoya K. 2004. Song syntax in Bengalese finches: proximate and ultimate analyses. Behavioral Sciences 34: 297–346.

Okanoya K. 2012. Behavioural factors governing song complexity in Bengalese finches. International Journal of Comparative Psychology 25: 44–59.

Olabarria C, Incera M, Garrido J, Rossi F. 2010. The effect of wrack composition and diversity on macrofaunal assemblages in intertidal marine sediments. Journal of Experimental Marine Biology and Ecology 396: 18–26.

Osiejuk TS. 2011. The song of the cinnamon-breasted bunting, *Emberiza tahapisi*, in the Bamenda Highlands (NW Cameroon). Journal of Ornithology 152: 651–659.

Otter K, Chruszcz B, Ratcliffe L. 1997. Honest advertisement and song output during the dawn chorus of black-capped chickadees. Behavioral Ecology 8: 167–173.

Otter KA, Ratcliffe L. 2005. Enlightened decisions: female assessment and communication networks. Animal communication networks. Cambridge University Press, Cambridge, UK.

Pagel MD. 1999. Inferring the historical patterns of biological evolution. Nature 401: 877–884.

Parker KA, Hauber ME, Brunton DH. 2010. Contemporary cultural evolution of a conspecific recognition signal following serial translocations. Evolution 64: 2431–2441.

Peek FW. 1972. An experimental study of the territorial function of vocal and visual display in the male red-winged blackbird (*Agelaius phoeniceus*). Animal Behaviour 20: 112–119.

Perrins C. 2009. The Princeton Encyclopedia of Birds. Princeton University Press, New Jersey, NJ, USA.

Petrie M, Kempenaers B. 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends in Ecology & Evolution 13: 52–58.

Petrie M, Doums C, Møller AP. 1998. The degree of extra-pair paternity increases with genetic variability. Proceedings of the National Academy of Sciences 95: 9390–9395.

Pfaff JA, Zanette L, MacDougall-Shackleton SA, MacDougall-Shackleton EA. 2007. Song repertoire size varies with HVC volume and is indicative of male quality in song sparrows (*Melospiza melodia*). Proceedings of the Royal Society of London B: Biological Sciences 274: 2035–2040.

Phillips JN, Derryberry EP. 2017. Vocal performance is a salient signal for male–male competition in White-crowned Sparrows. The Auk 134: 564–574.

Pinheiro J, Bates D, DebRoy S, Sarker D. 2013. R Development Core Team nlme: linear and nonlinear mixed effects models. R package version 3.1-111. Retrieved from: http://cran.r-project.org/web/ packages/nlme/index.html.

Podos J, Peters S, Rudnicky T, Marler P, Nowicki S. 1992. The organization of song repertoires in song sparrows: themes and variations. Ethology 90: 89–106.

Podos J, Lahti DC, Moseley DL. 2009. Vocal performance and sensorimotor learning in songbirds. Advances in the Study of Behavior 40: 159–195.

Poesel A, Foerster K, Kempenaers B. 2001. The dawn song of the blue tit *Parus caeruleus* and its role in sexual selection. Ethology 107: 521–531.

Poesel A, Dabelsteen T, Pedersen, SB. 2004. Dawn song of male blue tits as a predictor of competitiveness in midmorning singing interactions. Acta Ethologica 6: 65–71.

Poesel A, Kunc H, Foerster KM, Johnsen A, Kempenaers B. 2006. Early birds are sexy: male age, dawn song and extrapair paternity in blue tits, *Cyanistes* (formerly *Parus*) *caeruleus*. Animal Behaviour 72: 531–538.

Potvin DA, Crawford PW, MacDougall-Shackleton SA, MacDougall-Shackleton EA. 2015. Song repertoire size, not territory location, predicts reproductive success and territory tenure in a migratory songbird. Canadian Journal of Zoology 93: 627–633.

Pryke SR. 2013. Bird contests: from hatching to fertilisation. In: Hardy HCW, Briffa M (eds). Animal contests. Cambridge University Press, New York, NY, USA. pp. 287–303.

Purves EF, Conboy MA, Robertson RJ, Martin PR. 2016. Does white tail patch size indicate quality in male Cerulean warblers (*Setophaga cerulea*). The Wilson Journal of Ornithology 128: 343-354.

Price JJ, Friedman, NR, Omland, KE. 2007 Song and plumage evolution in the New World orioles (Icterus) show similar lability and convergence in patterns. Evolution 61: 850–863.

Quispe R, Sèbe F, da Silva ML, Gahr M. 2016. Dawn-song onset coincides with increased HVC androgen receptor expression but is decoupled from high circulating testosterone in an equatorial songbird. Physiology & Behavior 156: 1–7.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/

Read AF, Weary DM. 1992. The evolution of bird song: comparative analyses. Philosophical Transactions of the Royal Society B: Biological Sciences 338: 165–187.

Reichard DG, Rice RJ, Vanderbilt CC, Ketterson ED. 2011. Deciphering information encoded in birdsong: male songbirds with fertile mates respond most strongly to complex, low-amplitude songs used in courtship. The American Naturalist 178: 478–487.

Reichard DG, Rice RJ, Schultz EM, Schrock SE. 2013. Low-amplitude songs produced by male dark-eyed juncos (*Junco hyemalis*) differ when sung during intra- and inter-sexual interactions. Behaviour 150: 1183–1202.

Reid JM, Arcese P, Cassidy A, Hiebert SM, Smith JNM et al., 2004. Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. Animal Behavior 68: 1055–1063.

Reid JM, Arcese P, Cassidy ALEV, Hiebert SM, Smith JNM, Stoddard PK, Marr, AB, Keller LF. 2005. Fitness correlates of song repertoire size in free-living song sparrows (*Melospiza melodia*). The American Naturalist 165: 299–310.

Reid JM, Arcese P, Sardell RJ, Keller LF. 2011. Heritability of female extra-pair paternity rate in song sparrows (*Melospiza melodia*). Proceedings of the Royal Society of London B: Biological Sciences 278: 1114–1120.

Riters LV, Ball GF. 1999. Lesions to the medial preoptic area affect singing in the male European starling (*Sturnus vulgaris*). Hormones and Behavior 36: 276–286.

Ritchison G. 1988. Song repertoires and the singing behavior of male northern cardinals. Wilson Bulletin 100: 583–603

Ritschard M, Riebel K, Brumm H. 2010. Female zebra finches prefer high-amplitude song. Animal Behaviour 79: 877–883.

Robertson AW, Kelly D, Ladley JJ. 2011. Futile selfing in the trees *Fuchsia excorticata* (Onagraceae) and *Sophora microphylla* (Fabaceae): inbreeding depression over 11 years. International Journal of Plant Sciences 172: 191–198.

Rosvall KA, Reichard DG, Ferguson SM, Whittaker DJ, Ketterson ED. 2012. Robust behavioral effects of song playback in the absence of testosterone or corticosterone release. Hormones and Behaviour 62: 418–425.

Sandoval L, Mennill DJ. 2014. A quantitative description of vocalizations and vocal behaviour of the rusty-crowned ground-sparrow (*Melozone kienieri*). Ornitología Neotropical 25: 219–230.

Sanz JJ. 2001. Experimentally increased insectivorous bird density results in a reduction of caterpillar density and leaf damage to Pyrenean oak. Ecological Research 16: 387–394.

Sasahara K, Cody ML, Cohen D, Taylor CE. 2012. Structural design principles of complex bird songs: A network-based approach. PLOS ONE 7(9): e44436.

Schmidt R, Kunc HP, Amrhein V, Naguib M. 2008. Aggressive responses to broadband trills are related to subsequent pairing success in nightingales. Behavioral Ecology 19: 635–641.

Schmidt KL, Drew Moore S, MacDougall-Shackleton EA, MacDougall-Shackleton SA. 2013. Early-life stress affects song complexity, song learning and volume of the brain nucleus RA in adult male song sparrows. Animal Behaviour 86: 25–35.

Schmidt KL, MacDougall-Shackleton EA, Kubli SP, MacDougall-Shackleton SA. 2014. Developmental stress, condition, and birdsong: a case study in song sparrows. Integrative and Comparative Biology 54: 568–577.

Searcy WA, Andersson M. 1986. Sexual selection and the evolution of song. Annual Review of Ecology, Evolution, and Systematics 17: 507–533.

Searcy WA, Yasukawa K.1990. Use of the song repertoire in intersexual and intrasexual contexts by male red-winged blackbirds. Behavioral Ecology and Sociobiology 27: 123–128.

Searcy WA. 1992. Song repertoire and mate choice in birds. Integrative & Comparative Biology 32: 71–80.

Searcy WA, Yasukawa K. 1995. Polygyny and sexual selection in red-winged blackbirds. Princeton University Press, Princeton, NJ, USA.

Searcy WA, Yasukawa K. 1996. Song and female choice. In: Kroodsma DE, Miller EH (eds). Ecology and evolution of acoustic communication in birds. Cornell University Press, Ithaca, NY. pp. 454–473.

Searcy WA, Nowicki S, Hughes M. 1997. The response of male and female song sparrows to geographic variation in song. Condor 99: 651–657.

Searcy WA, Nowicki S. 2005. The evolution of animal communication: reliability and deception in signaling systems. Princeton University Press, Princeton, NJ, USA.

Secondi J, den Hartog PM, ten Cate C. 2002. To trill or not to trill? Territorial response to a heterospecific vocal trait in male collared doves, *Streptopelia decaocto*. Behavioral Ecology 14: 694–701.

Seddon N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical subpasserine birds. Evolution 59: 200–215.

Seddon N, Botero CA., Tobias JA, Dunn PO, MacGregor HEA, Rubinstein DR, Uy JA, Weir JT, Whittingham LA, Safran RJ. 2013. Sexual selection accelerates signal evolution during speciation. Proceedings of the Royal Society of London B: Biological Sciences 280: 20131065.

Senar JC, Burton PJK, Metcalfe NB. 1992. Variation in the nomadic tendency of a wintering finch *Carduelis spinus* and its relationship with body condition. Ornis Scandinavica 23: 63–74.

Sexton K, Murphy MT, Redmond LJ, Dolan AC. 2007. Dawn song of Eastern Kingbirds: intrapopulation variability and sociobiological correlates. Behaviour 144: 1273–1295.

Simpson RK, Johnson MA, Murphy TG. 2015. Migration and the evolution of sexual dichromatism: evolutionary loss of female coloration with migration among wood-warblers. Proceedings of the Royal Society of London B: Biological Sciences 282: 20150375.

Slagsvold T. 1977. Bird song activity in relation to breeding cycle, spring weather and environmental phenology. Ornis Scandinavica 8: 197–222.

Slagsvold T, Dale S, Sætre GP. 1994. Dawn singing in the great tit (*Parus major*): mate attraction, mate guarding, or territorial defence? Behaviour 131: 115–138.

Slater PJB. 2003. Fifty years of bird song research: a case study in animal behaviour. Animal Behaviour 65: 633–639.

Smith HG, von Schantz T. 1993. Extra-pair paternity in the European starling: the effect of polygyny. The Condor 95: 1006-1015.

Soma M, Garamszegi LZ. 2011. Rethinking birdsong evolution: meta-analysis of the relationship between song complexity and reproductive success. Behavioral Ecology 22: 363–371.

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). Hormones and Behavior 44: 132–139.

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. 2004. Developmental stress, social rank and song complexity in the European starling (*Sturnus vulgaris*). Proceedings of the Royal Society of London B: Biological Sciences 271: S121–123.

Spencer KA, Wimpenny JH, Buchanan KL, Lovell PG, Goldsmith AR, Catchpole CK. 2005. Developmental stress affects the attractiveness of male song and female choice in the zebra finch (*Taeniopygia guttata*). Behavioral Ecology and Sociobiology 58: 423–428.

Spencer KA, MacDougall-Shackleton SA. 2011. Indicators of development as sexually selected traits: the developmental stress hypothesis in context. Behavioral Ecology 22: 1–9.

Spottiswoode C, Møller AP. 2004. Extrapair paternity, migration, and breeding synchrony in birds. Behavioral Ecology 15: 41–57.

Stamps JA, Buechner M, Krishnan VV. 1987. The effects of habitat geometry on territorial defense costs intruder pressure in bounded habitats. American Zoologist 27: 307–325.

Stanley CQ, Walter MH, Venkatraman MX, Wilkinson GS. 2016. Insect noise avoidance in the dawn chorus of Neotropical birds. Animal Behaviour 112: 255–265.

Stewart AM, Craig JL. 1985. Movements, status, access to nectar, and spatial organisation of the tui. New Zealand Journal of Zoology 12: 649–666.

Suter SM, Ermacora D, Rielle N, Meyer DR. 2009. A distinct reed bunting dawn song and its relation to extrapair paternity. Animal Behaviour 77: 473–480.

Tacha TC, Vohs PA, Iverson GC. 1985. A comparison of interval and continuous sampling methods for behavioral observations. Journal of Field Ornithology 56: 258–264.

Taff CC, Steinberger D, Clark C, Belinsky K, Sacks H, Freeman-Gallant CR, Dunn PP, Whittingham LA. 2012. Multimodal sexual selection in a warbler: plumage and song are related to different fitness components. Animal Behaviour 84: 813–821.

Tarvin KA, Webster MS, Tuttle EM, Pruett-Jones S. 2005. Genetic similarity of social mates predicts the level of extrapair paternity in Splendid Fairy-wrens. Animal Behaviour 70: 945–955.

Taylor SS, Boessenkool S, Jamieson IG. 2008. Genetic monogamy in two long-lived New Zealand passerines. Journal of Avian Biology 39: 579–583.

Tchernichovski O, Nottebohm F, Ho CE, Pesaran B, Mitra PP. 2000. A procedure for an automated measurement of song similarity. Animal Behaviour 59: 1167–1176.

Trösch M, Müller W, Eens M, Iserbyt A. 2017. Genes, environments and their interaction: song and mate choice in canaries. Animal Behaviour 261–269.

ten Cate C. 2004. Birdsong and evolution. In: Marler PM., Slabbekoorn H (eds.). Nature's Music: the science of birdsong. Elsevier, San Diego, CA, USA. pp. 296–317.

Thomas RJ. 1999. Two tests of a stochastic dynamic programming model of daily singing routines in birds. Animal Behaviour 57: 277–284.

Titus RC. 1998. Short-range and long-range songs: use of two acoustically distinct song classes by dark-eyed juncos. The Auk 115: 386–393.

Tomialojč L, Lonkowski J. 1989. A technique for censusing territorial song thrushes Turdus philomelos. Annales Zoologici Fennici 26: 235–243.

Trillo PA, Vehrencamp SL. 2005. Song types and their structural features are associated with specific contexts in the banded wren. Animal Behaviour 70: 921–935.

Tu H-W, Severinghaus LL. 2004. Geographic variation of the highly complex hwamei (*Garrulax canorus*) songs. Zoological Studies 43: 629–640.

Tubaro PL, Segura ET, Handford P. 1993. Geographic variation in the song of the rufous-collared sparrow in Eastern Argentina. Condor 95: 588–595.

Tubaro PL, Segura ET. 1994. Dialect Differences in the Song of *Zonotrichia capensis* in the Southern Pampas: A Test of the Acoustic Adaptation Hypothesis. Condor 96: 1084–1088.

Turčoková L, Osiejuk TS, Pavel V, Glapan J, Petrusková T. 2010. Song divergence of two bluethroat subspecies. Ornis Fennica 87: 168–179.

Turjeman SF, Centeno-Cuadros A, Eggers U, Rotics S, Blas J, Fiedler W, Kaatz M, Jeltsch F, Wikelski M, Nathan R. 2016. Extra-pair paternity in the socially monogamous white stork (*Ciconia ciconia*) is fairly common and independent of local density. Scientific Reports 6: 27976.

Ulubay G. 2013. Resilient features of re-emerging dyadic communication systems in an interactive virtual environment [PhD] Graduate School of Informatics, Middle East Technical University, Ankara, Turkey.

Valderrama SV, Molles LE, Waas JR, Slabbekoorn H. 2013. Conservation implications of song divergence between source and translocated populations of the North Island Kōkako. Journal of Applied Ecology 50: 950–960.

Vallet E, Beme I, Kreutzer M. 1998. Two-note syllables in canary songs elicit high levels of sexual display. Animal Behaviour 55: 291–297.

Van Dongen WFD, Mulder RA. 2006. Habitat density, song structure and dialects in the Madagascar paradise flycatcher (*Terpsiphone mutata*). Journal of Avian Biology 37: 349–356.

Van Roo BL. 2004. Exogenous testosterone inhibits several forms of male parental behavior and stimulates song in a monogamous songbird: the blue-headed vireo (*Vireo solitarius*). Hormones and Behavior 46: 678–683.

Vargas-Rodriguez YL, Vazquez-Garcia JA, Williamson GB. 2005. Environmental correlates of tree and seedling-sapling distributions in a Mexican tropical dry forest. Plant Ecology 180: 117–134.

Ward S, Lampe HM, Slater PJ. 2004. Singing is not energetically demanding for pied flycatchers, *Ficedula hypoleuca*. Behavioral Ecology 15: 477–484.

Weir JT, Wheatcroft D. 2011. A latitudinal gradient in rates of evolution of avian syllable diversity and song length. Proceedings of the Royal Society of London B: Biological Sciences 278: 1713–1720.

Wells SJ. 2014. Evolutionary consequences and fitness correlates of extra-pair mating in the tui, *Prosthemadera novaeseelandiae* [PhD] Massey University, Auckland, New Zealand.

Wells SJ, Ji W, Dale J, Jones B, Gleeson D. 2015. Male size predicts extrapair paternity in a socially monogamous bird with extreme sexual size dimorphism. Behavioral Ecology 26: 200–206.

West MJ, King AP, Eastzer DH. 1981. Validating the female bioassay of cowbird song: relating differences in song potency to mating success. Animal Behaviour 29: 490–501.

Wiley RH, Richards DG. 1982. Adaptations for acoustical communication in birds: sound transmission and signal detection. In: Kroodsma DE, Miller EH (eds). Acoustic communication in birds Volume I: Production, perception and design features of sounds. Academic Press, Cambridge, MA, USA. pp. 131–181.

Wiley RH. 1991. Associations of song properties with habitats for territorial oscine birds of eastern North America. The American Naturalist 138: 973–993.

Woodgate JL, Leitner S, Catchpole CK, Berg ML, Bennett ATD, Buchanan KL. 2011. Developmental stressors that impair song learning in males do not appear to affect female preferences for song complexity in the zebra finch. Behavioral Ecology 22: 566–573.

Woodgate JL, Buchanan KL, Bennett ATD, Catchpole CK, Brighton R, Leitner S. 2013. Environmental and genetic control of brain and song structure in the zebra finch. Evolution 68: 230–240.

Woolley SMN, Rubel EW. 1997. Bengalese finches *Lonchura striata domestica* depend upon auditory feedback for the maintenance of adult song. Journal of Neuroscience 17: 6380–6390.

Woolley SC, Doupe AJ. 2008. Social context-induced song variation affects female behavior and gene expression. PLOS BIOL 6: e62.

Yasukawa K. 1981. Song repertoires in the red-winged blackbird (*Agelaius phoeniceus*): a test of the Beau Geste hypothesis. Animal Behaviour 29: 114–125.

Yu MS, Eng ML, Williams TD, Guigueno MF, Elliott JE. 2017. Assessment of neuroanatomical and behavioural effects of in ovo methylmercury exposure in zebra finches (*Taeniopygia guttata*). Neuro Toxicology 59: 33–39.

Yuta T, Koizumi I. 2016. Does nest predation risk affect the frequency of extra-pair paternity in a socially monogamous passerine? Journal of Avian Biology 47: 153–158.

Zann RA. 1996. The zebra finch: a synthesis of field and laboratory studies. Oxford University Press, Oxford, UK.

Zann R, Dunstan E. 2008. Mimetic song in superb lyrebirds: species mimicked and mimetic accuracy in different populations and age classes. Animal Behaviour 76: 1043–1054.

Zeng SJ, Székely T, Zhang XW, Lu K, Liu L, Zuo MX. 2007. Comparative analyses of song complexity and song-control nuclei in fourteen oscine species. Zoological Science 24: 1–9.

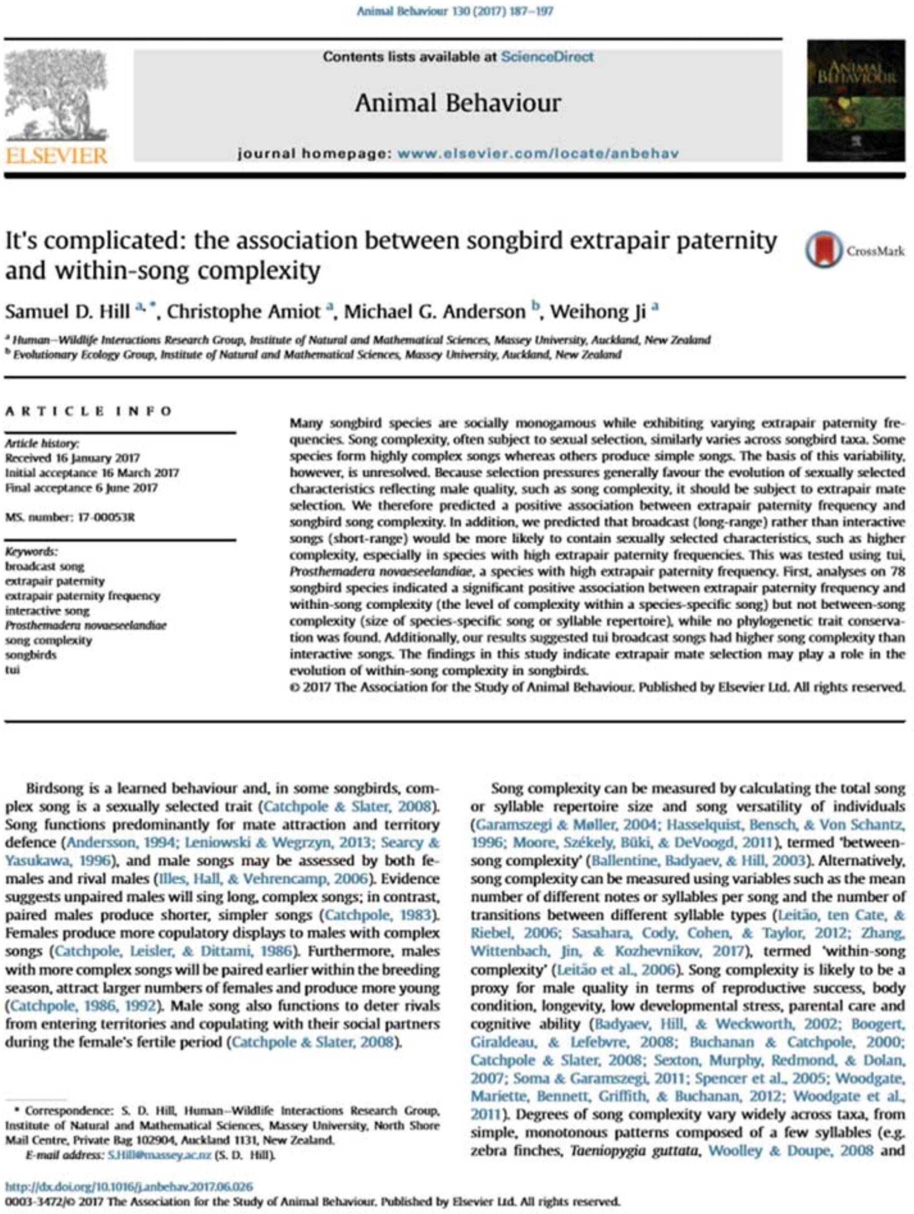
Zhang W, Hu C. 2016. Solar cell as wings of different sizes for flapping-wing micro air vehicles. International Journal of Micro Air Vehicles 8: 209–220.

Zhang Y, Wittenbach JD, Jin DZ, Kozhevnikov AA. 2017. Temperature manipulation in songbird brain implicates the premotor nucleus HVC in birdsong syntax. Journal of Neuroscience 37: 2600–2611.

Appendices

Tui in a manuka (*Leptospermum scoparium*) (photo: Jean Goodbrand, with permission).

Appendix 1: Reprints of published chapters and chapters in press



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It's complicated: the association between songbird extrapair paternity and within-song complexity

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Many songbird species are socially monogamous while exhibiting varying extrapair paternity frequencies. Song complexity, often subject to sexual selection, similarly varies across songbird taxa. Some species form highly complex songs whereas others produce simple songs. The basis of this variability, however, is unresolved. Because selection pressures generally favour the evolution of sexually selected characteristics reflecting male quality, such as song complexity, it should be subject to extrapair mate selection. We therefore predicted a positive association between extrapair paternity frequency and songbird song complexity. In addition, we predicted that broadcast (long-range) rather than interactive songs (short-range) would be more likely to contain sexually selected characteristics, such as higher complexity, especially in species with high extrapair paternity frequencies. This was tested using tui, *Prosthemadera novaeseelandiae*, a species with high extrapair paternity frequency. First, analyses on 78 songbird species indicated a significant positive association between extrapair paternity frequency and within-song complexity (the level of complexity within a species-specific song) but not between-song complexity (size of species-specific song or syllable repertoire), while no phylogenetic trait conservation was found. Additionally, our results suggested tui broadcast songs had higher song complexity than interactive songs. The findings in this study indicate extrapair mate selection may play a role in the evolution of within-song complexity in songbirds.

Birdsong is a learned behaviour and, in some songbirds, complex song is a sexually selected trait (Catchpole & Slater, 2008). Song functions predominantly for mate attraction and territory defence (Andersson, 1994; Leniowski & Wegrzyn, 2013; Searcy & Yasukawa, 1996), and male songs may be assessed by both females and rival males (Illes, Hall, & Vehrencamp, 2006). Evidence suggests unpaired males will sing long, complex songs; in contrast, paired males produce shorter, simpler songs (Catchpole, 1983). Females produce more copulatory displays to males with complex songs (Catchpole, Leisler, & Dittami, 1986). Furthermore, males with more complex songs will be paired earlier within the breeding season, attract larger numbers of females and produce more young (Catchpole, 1986, 1992). Male song also functions to deter rivals from entering territories and copulating with their social partners during the female's fertile period (Catchpole & Slater, 2008).

Song complexity can be measured by calculating the total song or syllable repertoire size and song versatility of individuals (Garamszegi & Møller, 2004; Hasselquist, Bensch, & Von Schantz, 1996; Moore, Székely, Büki, & DeVoogd, 2011), termed 'between-song complexity' (Ballentine, Badyaev, & Hill, 2003). Alternatively, song complexity can be measured using variables such as the mean number of different notes or syllables per song and the number of transitions between different syllable types (Leitão, ten Cate, & Riebel, 2006; Sasahara, Cody, Cohen, & Taylor, 2012; Zhang, Wittenbach, Jin, & Kozhevnikov, 2017), termed 'within-song complexity' (Leitão et al., 2006). Song complexity is likely to be a proxy for male quality in terms of reproductive success, body condition, longevity, low developmental stress, parental care and cognitive ability (Badyaev, Hill, & Weckworth, 2002; Boogert, Giraldeau, & Lefebvre, 2008; Buchanan & Catchpole, 2000; Catchpole & Slater, 2008; Sexton, Murphy, Redmond, & Dolan, 2007; Soma & Garamszegi, 2011; Spencer et al., 2005; Woodgate, Mariette, Bennett, Griffith, & Buchanan, 2012; Woodgate et al., 2011). Degrees of song complexity vary widely across taxa, from simple, monotonous patterns composed of a few syllables (e.g. zebra finches, *Taeniopygia guttata*, Woolley & Doupe, 2008 and

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European warblers, *Acrocephalus* spp., Catchpole, 1980) to highly complex songs with a large syllable diversity, organized in nonrandom fashion with discernible patterns (e.g. European starlings, *Sturnus vulgaris*, Moorman & Bolhuis, 2013 and California thrasher, *Toxostoma redivivum* Sasahara et al., 2012). Notwithstanding, song complexity variation also occurs within taxonomic families. In the honeyeaters (Meliphagidae) for example, the repertoires of noisy miners, *Manorina melanocephala*, and New Holland honeyeaters, *Phylidonyris novaehollandiae*, consist of simple single high-frequency notes (Jurisevic & Sanderson, 1994). Tui, *Prosthemadera novaeseelandiae*, on the other hand, sing highly complex songs consisting of many different syllables (Hill, 2011; Hill, Amiot, Ludbrook, & Ji, 2015). The evolutionary basis of phenotypic songbird variation in song complexity levels is somewhat of a conundrum to evolutionary ecologists (Kaluthota, 2016).

Extrapair paternity (EPP) can be defined as 'deviations from genetic monogamy' (Turjeman et al., 2016), where males sire offspring outside their social bond (e.g. Forstmeier, Nakagawa, Griffith, & Kempenaers, 2014). In songbirds, EPP is often directed by a female which will initiate extrapair (EP) mating (Bowers et al., 2015). Extrapair paternity is widespread across songbird taxa (Taylor, Boessenkool, & Jamieson, 2008) despite many species being socially monogamous (Hasselquist & Sherman, 2001; Liu et al., 2015). Extrapair copulations are more likely to occur in species where biparental care is of low importance to chick survival (Petrie & Kempenaers, 1998). Females may attain direct benefits from EP copulations. For example, they may be able to feed on an EP partner's territory, in addition to having EP offspring with lower depredation and starvation rates (Gray, 1997). Moreover, EP mating enhances the inclusive fitness of females beyond the genetic capability of their social partner (Bowers et al., 2015). This is reflected in the increased additive genetic value of offspring, often referred to as the 'good genes hypothesis' (see Reid, Arcese, Sardell, & Keller, 2011). Offspring of EP males may also have greater heterozygosity (Foerster, Delhey, Johnsen, Lifjeld, & Kempenaers, 2003).

Extrapair paternity frequency varies within taxa (Spottiswoode & Møller, 2004) and across taxa (Canal, Jovani, & Potti, 2012). For example, previous studies suggest EPP frequency in the Savi's warbler, *Locustella luscinioides*, is 4.1% of all offspring observed (Neto, Hansson, & Hasselquist, 2010) whereas in superb fairy-wrens, *Malurus splendens*, 65% of offspring are a result of EPP (Tarvin, Webster, Tuttle, & Pruett-Jones, 2005). Furthermore, EPP frequency in the great reed warbler, *Acrocephalus arundinaceus*, is 4% (Hasselquist, Bensch, & Vonschantz, 1995) and in a tui population, 57% of offspring observed resulted from EPP (Wells, Ji, Dale, Jones, & Gleeson, 2015).

Selection pressures generally favour the evolution of sexually selected characteristics reflecting male genetic quality (e.g. Garamszegi & Møller, 2004; Kempenaers et al., 1992). This is particularly evident in species with high EPP frequencies, as these males usually do not provide parental care (Møller, 2000). We would therefore predict that song complexity would be more pronounced in species with high EPP frequencies, as males of these species would be under more intense sexual selection pressures. In other words, there would be a significant positive association between EPP frequency and song complexity in songbird taxa. Some evidence supports this prediction and indeed females have been found to select EP males with larger song repertoires and higher levels of song consistency (Hasselquist et al., 1995; Byers, 2007; Read & Weary, 1992). However, other studies including meta-analyses of 65 (Garamszegi & Møller, 2004) and 27 (Soma & Garamszegi, 2011) songbird species found no significant relationship between EPP and multiple song features. Therefore, whether male song traits play a role in EPP mate selection remains uncertain. We tested the prediction that EPP frequency was positively associated with song complexity in multiple songbird species. To do this, we employed both within- and between-song complexity measures in addition to a larger sample size of 78 species. Advancing from the above-mentioned meta-analyses, we also extracted and analysed song complexity data from song recordings both from the above-mentioned databases, and from field recordings of tui and yellowhammer, *Emberiza citrinella*.

Songbird songs have been categorized into two classes according to audience and transmission range: long-range broadcast songs (BS) and short-range interactive songs (IS; Nolan et al., 2002; Titus, 1998; Reichard, Rice, Schultz, & Schrock, 2013; Rosvall, Reichard, Ferguson, Whittaker, & Ketterson, 2012). Broadcast songs are defined as prominent and nondirectional vocal signals, functioning as advertisements of male reproductive and territorial status (Titus, 1998; Wiley & Richards, 1982). They are directed to no obvious audience; therefore, they probably play an important role in attracting both social and EP mates. Interactive songs, on the other hand, are often initiated and directed towards an observable audience. They are employed in male–male confrontations (Titus, 1998), in addition to courtship and pair bond maintenance (Reichard, Rice, Vanderbilt, & Ketterson, 2011; Titus, 1998). If song complexity is selected in EP mating, BS should contain high complexity in species with high EPP frequencies. Little information is available regarding differences in song classes in the context of mate selection in songbirds.

Previous research suggested IS in the dark-eyed junco, *Junco hyemalis*, a species with moderate EPP frequency (27%, Gerlach, McGlothlin, Parker, & Ketterson, 2012), are more complex than BS in terms of syllable diversity. Using tui, a species with both very high EPP frequency and song complexity, we investigated the role of BS within the context of mate attraction and examined whether BS differ in complexity to IS. We predicted that BS would contain higher levels of within-song complexity than IS.

METHODS

Data Collection: EPP and Song Complexity in Multiple Species

Breeding season songs of males of 78 songbird species were collected, 76 of which were obtained exclusively from online databases: Macaulay Library archive of wildlife sounds and videos of Cornell Lab of Ornithology (Cornell University; www.macaulaylibrary.org) and the Xeno-Canto citizen science project website (www.xeno-canto.org). Songs were extracted from 175 recording files from the Macaulay Library, 53 from Xeno-Canto and four from the field (tui and yellowhammer, Tawharanui Regional Park, New Zealand, using a Marantz PMD620 Solid-State Digital Recorder [Marantz, Kanagawa, Japan], attached to a Sennheiser ME67 shotgun long-range directional microphone [Sennheiser, Old Lyme, CT, U.S.A.]). Xeno-Canto only supports mp3 files and these were converted to wav files using http://www.audio.online-convert.com/convert-to-wav. The Xeno-Canto archive has been used to provide compressed sound files in other studies analysing and examining avian song structure (Weir & Wheatcroft, 2011) without having any significant impact on song structural integrity. However, we acknowledge that the recording quality on these files may have been marginally lower than for the other sound samples used in this study. This is, however, unlikely to have had any significant effect on analyses within this study.

All species analysed within our study included 64 of the 65 analysed in a previous meta-analysis by Garamszegi and Møller (2004). The remaining 14 species were chosen to obtain a wider range of EPP frequency and to increase sample size from this previous study. Multiple species within this data set have socially monogamous mating systems with varying EPP frequencies (see

Supplementary material). The EPP frequency data for each species were obtained from research articles found in the Google Scholar (Mountainview, CA, U.S.A., www.scholar.google.com) and Web of Science (Clarivate Analytics, U.S.A., www.clarivate.com/?product=web-of-science) databases, in addition to research articles utilized by Garamszegi and Møller (2004) and Soma and Garamszegi (2011) to gather EPP data. Some published articles were the only research papers available on specific species. Some species within this data set have also been noted as exhibiting polygamous (a male or female having more than one social partner, Hasselquist et al., 1995) or cooperative breeding behaviour (alloparental offspring care, where parental care of young is augmented by other group members, Piper & Slater, 1993).

When songs from multiple individuals of the same species were available, a single randomly selected song was chosen from each of three randomly selected individuals (total of 232 individuals); in one species, Henderson reed warbler, *Acrocephalus taiti*, only one male recording was available. Only recordings with the highest quality rating in each database were used (i.e. those with a good signal-to-noise ratio). The time, date, location, context, whether songs were natural or induced using playback (all songs used in analyses were natural), habitat (we maintained intraspecific habitat consistency in our choice of recordings where possible) and comprehensive details of each song in terms of their song class (i.e. BS or IS) were available and extracted from the database. Only BS were used in this part of the study to avoid any confounding factors because differences in BS and IS structure and complexity have been noted as described. Furthermore, the most common song types available were BS. Analysis of the differences in complexity between BS and IS was conducted in a high EPP species, tui (details later within this section).

Data Collection: Song Complexity Variation in Tui

Tui are endemic New Zealand songbirds within the family Meliphagidae (Robertson, Kelly, & Ladley, 2011). They are socially monogamous with high EPP frequency (57%, Wells et al., 2015), high sexual size dimorphism (Wells et al., 2015), and exhibit highly agonistic behaviour (Craig, 1984). Tui have high song complexity and a large song and syllable repertoire (Hill & Ji, 2014), which are likely to be products of sexual selection.

Song data from adult male tui were collected from 11 different rural locations across New Zealand's North Island and nearby offshore islands (Fig. 1a and b, Table 1). Data collection was carried out over four breeding seasons (October–February 2010/2011, 2011/2012, 2012/2013 and 2013/2014). During sampling sessions, we walked slowly along marked tracks to locate tui at all recording sites. When a tui was located, recording was initiated and ended when it either ceased singing or departed its singing perch (the total sampling effort per individual varied: minimum: 1.8 min; maximum: 12.9 min). Recording, however, was reinitiated if the same individual started singing again within 5 min of cessation. During each recording, the location, time, the band combination of the tui (if applicable), behaviour and sex were noted. Broadcast songs were identified as such when tui projected songs from high vantage points to no immediately observable audience. Interactive songs were identified as such when directed, at close range, towards another individual, either male or female.

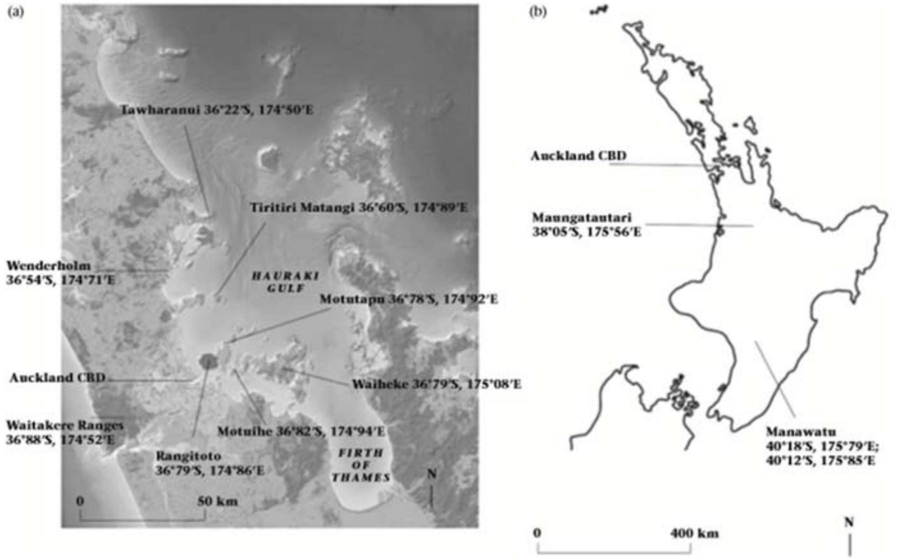

Figure 1. The location of the eight study sites around the Auckland region including (a) the five offshore islands (map from the National Institute of Water and Atmospheric Research, with permission) and (b) the location of the three mainland study sites in relation to Auckland's central business district.

Table 1
Tui song recording sites and number of tui recorded

Location	Size (ha)	Endemic songbird species present	Number of tui sampled
Ark in the Park, Waitakere	1100	*Calleaus wilsoni, Gerygone igata,* *Petroica longipes, Petroica macrocephala toitoi, Rhipidura fuliginosa*	3
Maungatautari southern enclosure	65	*Anthornis melanura, Gerygone igata,* *Mohoua albicilla, Notiomystis cyncta, Petroica longipes,* *Petroica macrocephela toitoi, Philesturnus rufusater, Rhipidura fuliginosa*	3
Motuihe	179	*Gerygone igata, Mohoua albicilla, Philesturnus rufusater, Rhipidura fuliginosa*	2
Motutapu	1510	*Anthornis melanura, Gerygone igata, Philesturnus rufusater, Rhipidura fuliginosa*	1
Pohangina wetlands	7.2	*Anthornis melanura, Gerygone igata, Rhipidura fuliginosa*	1
Rangitoto	2311	*Anthornis melanura, Gerygone igata, Mohoua albicilla,* *Petroica macrocephela toitoi, Philesturnus rufusater, Rhipidura fuliginosa*	2
Tawharanui Regional Park	588	*Anthornis melanura, Gerygone igata Mohoua albicilla,* *Petroica longipes Philesturnus rufusater*	28
Tiritiri Matangi	254	*Acanthisitta chloris, Anthornis melanura, Calleaus wilsoni,* *Gerygone igata, Mohoua albicilla, Petroica longipes,* *Philesturnus rufusater, Rhipidura fuliginosa*	3
Totara Reserve, Pohangina	340	*Anthornis melanura, Gerygone igata, Rhipidura fuliginosa*	1
Victoria Reserve, Waiheke	11	*Gerygone igata, Rhipidura fuliginosa*	3
Wenderholm Regional Park	134	*Gerygone igata, Rhipidura fuliginosa*	4

Among the 28 males we recorded at Tawharanui Regional Park, 17 were individually marked with colour bands. For each banded bird, we only analysed songs that were recorded in a single session. For unbanded birds, only one unbanded individual was recorded at any one time and in any one identified territory in each location to minimize the chances of pseudoreplication. During the breeding season when tui are paired and have nests, both nests and flowering plants are guarded, and tui remain within their respective territories. Furthermore, males also tend to sing on specific perches (Hill, Ji, Parker, Amiot, & Wells, 2013) which further minimized the chances of pseudoreplication. All songs included in the analysis were recorded between 0600 and 1500 hours at 2–5 m from each bird. Only complete songs with good signal-to-noise ratios were included in analyses.

A Marantz PMD620 Solid-State Digital Recorder was used to record songs, attached to a Sennheiser ME67 shotgun long-range directional microphone. All songs were recorded at a sampling frequency of 44.1 kHz and at a resolution of 16 bits. The frequency response range of the long-range microphone was 40 to 20 000 Hz. Recordings were digitized using Raven Pro 1.4 Beta Version software (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.) with the same settings as described earlier.

Song Variables and Data Analysis for Multiple Species

To assess the relationship between EPP frequency and both within- and between-song complexity, linear regressions were employed (SPSS, version 23.0; IBM, Chicago, IL, U.S.A.). Four variables measuring within-song complexity were extracted from each song: number of syllables, song duration, syllable diversity and syllable transitions (Table 2) using Raven Pro. Evidence suggests these four song complexity variables within male BS are assessed and sexually selected by females, and are therefore are important in mate attraction (Boogert et al., 2008). An overall within-song complexity score was then created by adding together the values for each of these four complexity variables for each individual (adapted from the methodology described by Ulubay, 2013).

We also extracted three key between-song complexity variables, song and syllable repertoire size and song versatility from the same recordings, as above to maintain consistency (Table 2). Song and syllable repertoire size were quantified by tallying the number of unique songs and syllables produced by each individual in each total recording. The number of songs, however, varied for each recording; therefore, the song repertoire size score for each

Table 2
Song variables employed in this study to compare song complexity in 78 different songbird species with varying extrapair paternity frequencies, and broadcast and interactive song in tui

Song variable	Definition
Number of syllables	The total number of sound units or sound unit complexes (syllables) per song. A note alone without a repeat was considered a syllable. When multiple identical notes were repeated consecutively, this cluster was also considered a single syllable (Boogert et al., 2008)
Song duration (s)	The length of time from the start of a note or syllable to the end of a note or syllable in a series of syllables preceded and followed by a ≥3 s interval (Kroodsma, 1977)
Song repertoire size	The number of distinct songs within the repertoire of an individual (Gil & Slater, 2000). Only used for the multiple species analysis
Song versatility	Measures the amount of switching from one song type to another within an individual's singing bout (Garamszegi & Møller, 2004). Only used for the multiple species analysis
Syllable diversity	The number of different sound units or sound unit complexes (syllables) per song. Syllables can comprise several notes (Boogert et al., 2008)
Syllable repertoire size	The number of distinct syllables within the repertoire of an individual (Moore et al., 2011). Only used for the multiple species analysis
Syllable transitions	The number of syllable switches per song (Feßl & Hoi, 2000)
Trill duration (s)	Cumulative total length of repeated, high-frequency modulation portions per song (Hill et al., 2015). Only used for the tui song class analysis
Trill percentage	Percentage of each song that contains rapid, repeated, high-frequency modulation notes. Computed by dividing song duration with the amount of time (s) trills are produced within a song (Hill et al., 2013). Only used for the tui song class analysis

individual was based upon the percentage of songs recorded that were unique within that individual's repertoire (i.e. the number of unique song types produced by an individual was divided by the total number of songs recorded for that individual and then that number was multiplied by 100 [adapted from Podos, Peters, Rudnicky, Marler, & Nowicki, 1992]). To measure song versatility, songs were scored as being versatile (score of two) if song types were switched rapidly; eventually versatile (score of one) if song types were repeated within a repertoire before eventually switching; and nonversatile (score of zero) if a song type was repeated continuously within a repertoire (as described by Garamszegi & Møller, 2004). We excluded from the between-song complexity analysis house sparrow, *Passer domesticus*, as their songs only consisted of one syllable and distinguishing between songs was not possible, and Henderson reed warbler, for which only one recorded song was available for analysis. All song values used within this part of the study were averaged for each species for all statistical analyses. All song complexity scores were log-transformed prior to linear regression analyses as they were nonparametric in distribution, as determined by Kolmogorov–Smirnov tests (using SPSS).

For all recordings, spectrograms were created by discrete Fourier transform (DFT) with a Hann window. The frame length was set at 256 points. Additionally, a 50% frame overlap with a hop size of 2.9 ms was used. A frequency grid spacing of 172 Hz was also employed and the bandwidth was set at 3 dB.

Songs from each species were analysed blind to their respective EPP frequencies to avoid potential sampling bias. Recordings (written as wav files at a sampling rate of 44.1 kHz and at a resolution of 16 bits) were digitized and song variables measured using Raven Pro. A song was defined as a period of continuous syllables surrounded, before and after, by a silent intersong interval period of ≥ 3 s (Woolley & Rubel, 1997) and only complete songs were analysed. All 78 species analysed produced discontinuous songs, or songs that had discernible intersong intervals.

Phylogenetic correction

Comparative analyses examining ecological differences across taxa need to consider potential effects due to shared ancestry (Lifjeld, Laskemoen, Kleven, Albrecht, & Robertson, 2010). A phylogenetic tree for these 78 species was created using 'a global phylogeny of birds' (available from: http://birdtree.org) based on the most comprehensive studies (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012, 2014). An image of the phylogenetic tree from the resulting file was created using FigTree (version 1.4.2, Fig. 2). The branch length units are based on nucleotide substitutions per site, i.e. the number of changes or 'substitutions' divided by the length of the sequence. We used phylogenetic generalized least squares (PGLS) for phylogenetic correction to determine whether phylogeny significantly influenced the linear regression analysis (see Barger, Hanson, Teffer, Schenker-Ahmed, & Semendeferi, 2014) used to test for associations between EPP frequency and song complexity. The PGLS estimates the value of lambda (λ, Pagel, 1999) to estimate phylogenetic correlation. If $\lambda =$ '1', the diversification ratio is phylogenetically conserved; conversely, if $\lambda =$ '0' this implies the diversification ratio is random with respect to phylogeny (i.e. the traits are independent of phylogeny, in other words, there is more divergence between taxa than expected under a Brownian motion model of trait evolution). The PGLS analysis was carried out using the nlme package (Pinheiro, Bates, DebRoy, & Sarker, 2013) in R (version 2.15.3). For model selection, we used the second-order Akaike information criterion (AIC, Burnham & Anderson, 2002) and likelihood ratio (LR) tests. The PGLS estimated λ for the model and tested whether it was significantly different from zero.

Mating system

Mating system is associated with sperm competition in songbirds, which is widespread in socially monogamous species that undertake extrapair copulations (Birkhead, 1998). We therefore controlled for mating system in the comparative analyses of the 78 songbird species. To test whether the different mating systems described above (categorized as: (1) socially monogamous, (2) polygamous or (3) cooperative extracted from relevant literature [see Supplementary material]) were linked to EPP frequency, in the multiple species, a multiple regression test was conducted. We also conducted a multiple regression test aiming to detect any association between mating system and the overall within-song complexity score.

Sexual dichromatism

We also controlled for sexual dichromatism, variation in plumage colour, in all 78 species (as per Garamszegi & Møller, 2004; but see Huang & Rabosky, 2015) as this has been linked with variation in songbird EPP frequency. Garamszegi and Møller (2004) suggested that of possible confounding factors, only sexual dichromatism had a statistically significant relationship with EPP frequency. There is no evidence that aspects such as habitat or migration had a relationship with EPP frequency. Therefore, these factors were not included in this analysis in terms of potential confounding variables. Sexual dichromatism was categorized on a scale (zero intersexual difference in plumage (scored 0) to male with full colour and the female brown (5)) based on species descriptions (Perrins, 2009). A multiple regression test was conducted to ascertain whether sexual dichromatism had a relationship with EPP frequency in the multiple study species. We also conducted a multiple regression test to try to detect any association between sexual dichromatism and the overall song complexity score described above.

Parental care

We controlled for parental care (e.g. rate of nestling feeding) for all 78 species by testing for correlations between parental care and EPP frequency. Parental care for all species was categorized as (1) female only, (2) male only, (3) combination of male and female or (4) communal feeding according to relevant literature (see Supplementary material). A multiple regression test was conducted to ascertain whether parental care had a relationship with EPP frequency in the multiple study species. We also conducted a multiple regression test to try to detect any association between parental care and the overall song complexity score. All analyses were conducted using SPSS.

Song Variables and Data Analysis for Tui Song Classes

To compare tui song complexity between BS and IS, one song per class per individual was included in analyses, if indeed both song classes were produced during the recording session. A single song was chosen from each individual and song class at random. For three tui, no IS recordings were obtained. The four within-song complexity variables used for the across-taxa analyses were extracted for this part of the analysis. Two additional within-song complexity variables were also used (trill duration and trill proportion). These two variables are consistent characteristics of tui vocalizations but are not produced by all songbird species which is the reason it was not used for the across-taxa song analysis. This was conducted using Raven Pro (Fig. 3a and b, Table 2; Hill et al., 2013). Between-song complexity analyses were not conducted in the tui song class analysis because there was an insufficient number of IS available for a comparative analysis with BS.

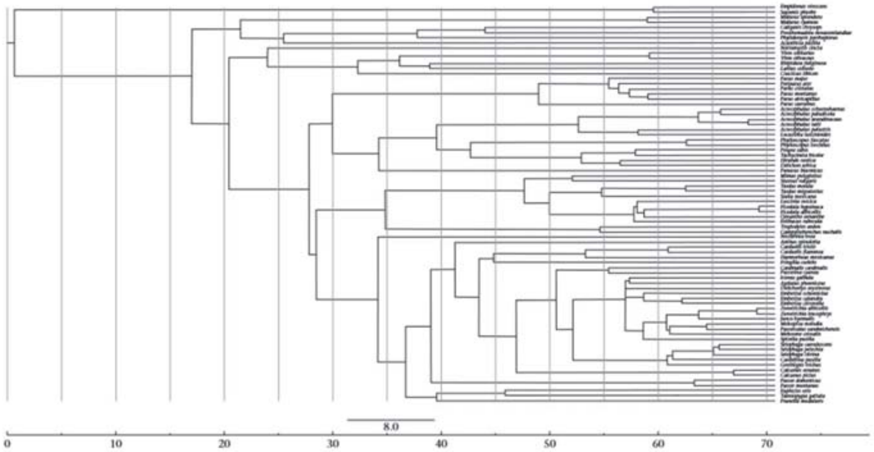

Figure 2. The phylogenetic tree of the 78 songbird species within this study, including the scale. The units of branch length are nucleotide substitutions per site, i.e. the number of changes or 'substitutions' divided by the length of the sequence.

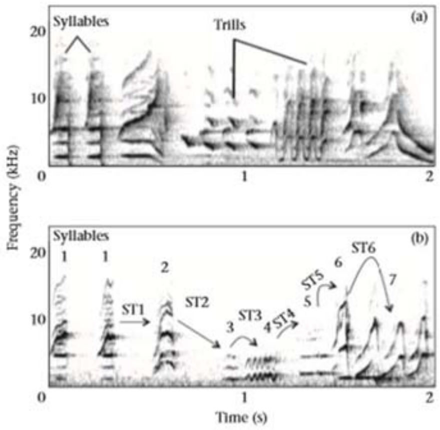

Figure 3. Spectrographic representation of the variables employed to measure song complexity in this study. Spectrograms show (a) a male tui broadcast song, including trills, which were only used in the analysis of tui song classes as they are a consistent feature of tui songs, and (b) a tui broadcast song showing how the number of syllable transitions (ST) were calculated for each song across all species.

Within-group Multivariate Analysis

All song complexity data were log-transformed to remove skewness and converted to Z-scores. Z-scores are used to standardize data from different distributions to provide a method of comparing them regardless of their respective distributions (Abdi, 2007). Different IS types were produced, male to a male, male to a small group of males and male to a female. A one-way analysis of similarity (ANOSIM, Clarke & Warwick, 1994) was used to detect whether song complexity between these different IS types was significantly dissimilar across samples. ANOSIM is a nonparametric multivariate test based on, in this instance, Euclidean distance measure, using the rank order of dissimilarity values to test whether song complexity using all six variables differed between IS types. A one-way permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) based on Euclidean distance measure was also used to test whether within-song complexity using all six variables differed between IS types. These analyses were conducted prior to multivariate comparisons between BS and IS. This allowed us to ascertain whether different IS could be pooled before comparisons with BS.

Between-group Multivariate Analysis

The variables from both BS and IS were then examined by one-way ANOSIM and PERMANOVA tests to detect whether song complexity between BS and IS was significantly dissimilar. A nonmetric multidimensional scaling (NMDS) ordination plot was then generated to provide a visual summary of the patterns of Euclidean values among the samples. All ANOSIM and PERMANOVA tests were conducted in conjunction with the conservative Bonferroni correction procedure for multiple hypothesis testing. The ANOSIM, PERMANOVA and NMDS analyses were all conducted using PAST software (version 2.17b; Hammer, Harper, & Ryan, 2001).

Table 3
A summary of the linear regression scores for the relationship between EPP frequency and each of the song complexity parameters

	R^2	EPP frequency t	P
Number of syllables per song	0.03	1.89	0.06
Overall within-song complexity	0.07	2.58	0.012
Song duration	0.02	1.59	0.116
Song repertoire size	0.06	0.65	0.521
Song versatility	0.03	−1.55	0.13
Syllable diversity	0.1	4.99	0.003
Syllable repertoire size	0.02	1.56	0.12
Syllable transitions	0.1	4.54	0.005

EPP: extrapair paternity.

RESULTS

EPP and song Complexity in Multiple species

A linear regression analysis indicated there was a statistically significant positive relationship between EPP frequency and the overall within-song complexity score in multiple species ($N = 78$) in addition to syllable diversity and the number of syllable transitions per song, but weaker associations with the number of syllables per song and song duration (Table 3). This suggests that EPP frequency predicted overall within-song complexity in the sampled data set of 78 songbird species (Fig. 4, Table 3).

A linear regression analysis also indicated there was no significant relationship between EPP frequency and between-song complexity parameters song repertoire size and song versatility (Table 3).

Phylogenetic Correction

We conducted a PGLS analysis to correct for phylogenetic relatedness for EPP frequency and song complexity variables. The PGLS estimated λ to be not significantly different from zero for the song complexity traits that exhibited a significant association with EPP in the linear regression analysis (Table 4). This suggests that the traits and the observed association between traits were independent of phylogeny and showed greater divergence between taxa than would be expected under a Brownian model of trait evolution.

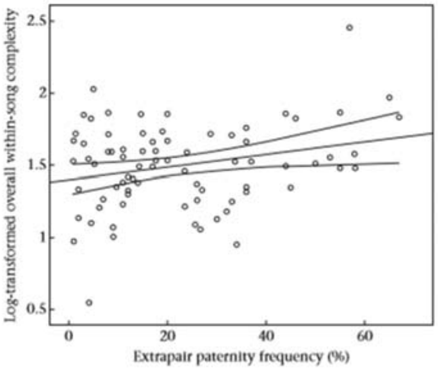

Figure 4. The relationship (nonphylogenetic linear regression with mean ± 95% confidence intervals) between extrapair paternity frequency and overall within-song complexity (log-transformed).

Table 4
The relationship between EPP frequency and the song complexity variables that exhibited a significant association with EPP frequency in a linear regression analysis, tested using phylogenetic generalized least squares analysis

Song variable		EPP frequency	
	Pagel's lambda λ	AIC	P
Overall within-song complexity	0.61	766.33	0.24
Syllable diversity	0.73	572.8	0.17
Syllable transitions	0.34	468.8	0.32

EPP: extrapair paternity; AIC: Akaike's information criterion. The P values here signify that the Pagel's λ values for these variables were not significantly different from zero. This suggests that these traits showed greater divergence between taxa than would be expected under a Brownian motion model of trait evolution (i.e. these traits evolved independent of phylogeny).

Mating System, Sexual Dichromatism and Parental Care

A multiple regression analysis indicated there was no significant association between EPP frequency and mating system ($N = 78$, $R^2 = -0.03$, $P = 0.065$), sexual dichromatism ($N = 78$, $R^2 = -0.03$, $P = 0.553$) and parental care ($N = 78$, $R^2 = -0.03$, $P = 0.157$).

A multiple regression analysis also indicated there was no significant association between the overall song complexity score and mating system ($N = 78$, $R^2 = 0.01$, $P = 0.338$), sexual dichromatism ($N = 78$, $R^2 = 0.01$, $P = 0.455$) and parental care ($N = 78$, $R^2 = 0.01$, $P = 0.218$). These results suggested that mating system, sexual dichromatism and parental care were not confounding variables for this study.

Tui Song Classes: Within-group (tui IS) Comparison

Comparison between male–male IS ($N = 27$) and male–female IS ($N = 18$) yielded no significant differences for all six variables (ANOSIM: $R = -0.042$, $P = 0.729$; PERMANOVA: pseudo $F_{38,45} = 2.353$, $P = 0.092$). All IS data were subsequently pooled for comparison with BS.

Comparison Between Tui BS and IS

There was a significant overall difference between BS and IS of male tui using all six variables measuring within-song complexity (ANOSIM: $N = 48, 45$, $R = 0.409$, $P < 0.001$ with Bonferroni correction, 9999 permutations; PERMANOVA: $N = 48, 45$, pseudo $F_{44,51} = 27.38$, $P < 0.001$ with Bonferroni correction, 9999 permutations; Fig. 5, Table 5). Broadcast songs had higher song complexity than IS.

DISCUSSION

We assessed the EPP frequency and song complexity associations in a wide range of songbird taxa with varying degrees of EPP. Our results indicated a significant positive association in multiple taxa between EPP frequency and overall within-song complexity but not with any between-song complexity variables. Species with higher EPP frequency produced more complex songs overall. In other words, our findings suggest complex vocal traits were ostensibly more pronounced in songbird species with higher EPP frequency. Although more work is still needed to resolve the biological relevance of this statistically significant relationship, our results suggest that EP mate selection may play a role in within-song complexity evolution.

Previous studies have suggested that aspects of song such as large individual song repertoires, song rate and song consistency, in addition to the ability to produce and maintain high sound amplitudes, are positively associated with EPP frequency (Forstmeier, Kempenaers, Meyer, & Leisler, 2002; Hasselquist et al., 1996; Suter, Ermacora, Rielle, & Meyer, 2009; Taff et al., 2012). Higher song complexity in BS may have therefore partly evolved to maximize the chances of obtaining both social mates and multiple extrapair mates. However, a previous study employing a meta-analysis found no relationship between songbird song complexity and EPP frequency (Garamszegi & Møller, 2004). These conclusions were supported to some degree by Soma and Garamszegi (2011) who stated multiple biological factors needed to be considered to fully understand the role song plays in reproductive success, such as differences in breeding systems between species. Our study revealed significant associations between EPP frequency and a specific key aspect of song complexity (within-song complexity). Within-song complexity is perhaps more useful in measuring the organization of song components (Sasahara et al., 2012) than a mere calculation of repertoire size or versatility. For example, some

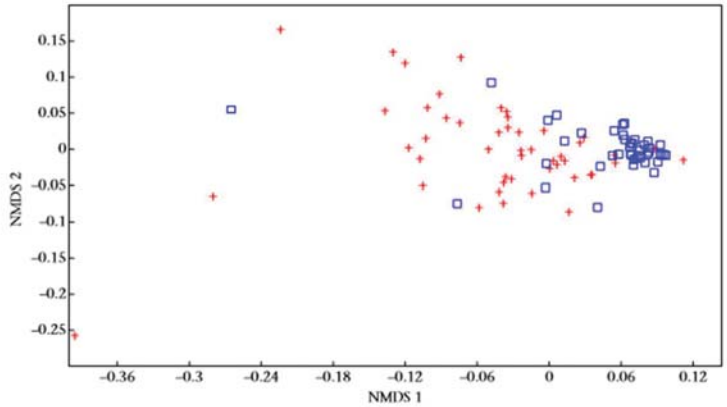

Figure 5. The nonmetric multidimensional scaling (NMDS) ordination plot based on Euclidean distance of broadcast song (red crosses) and interactive song (blue squares) in tui.

Table 5
Differences in within-song complexity parameters between broadcast and interactive song of male tui

Variable	Mean±SE	
	Broadcast song	Interactive song
Number of syllables	37±3	21±2
Song duration (s)	10.09±1.15	5.2±0.49
Syllable diversity	23±2	12±1
Syllable transitions	21±2	10±1
Trill duration (s)	4.59±0.86	1.23±0.36
Trill percentage (%)	44±3	16±3

species such as chaffinches, *Fringilla coelebs*, sing two to three basic song types (i.e. a small song repertoire). However, within these song types, differences in complexity can be substantial (i.e. high song complexity) despite each song having the same basic pattern (Leitão et al., 2006). Furthermore, higher within-song complexity can elicit stronger responses in both males and females suggesting it evolved under both inter- and intrasexual selection pressures (Leitão et al., 2006).

Within our study, there was no evidence of a significant association between EPP frequency and between-song complexity. One constraint of this aspect of our study, however, was the limited number of songs available and analysed per individual although an average was obtained for each species for each variable. Nevertheless, previous research in concordance with our study found neither ubiquitous nor compelling evidence of female sexual selection for larger song repertoires; rather, the authors suggested the relationship between song and reproductive success evolved as a by-product of social conditions favouring the evolution of complex signals (Byers & Kroodsma, 2009). Other studies found that female sedge warblers, *Acrocephalus schoenobaenus*, select EP males with smaller repertoires than their social partners (Marshall, Buchanan, & Catchpole, 2007). Conversely, some studies have indicated that large song repertoires are associated with greater reproductive success and therefore help attain direct fitness benefits. For example, male song sparrows, *Melospiza melodia*, and willow warblers, *Phylloscopus trochilus*, with large song repertoires had greater reproductive success than those with smaller repertoires (Catchpole, 1986; Gil & Slater, 2000), although larger repertoires have been positively associated with breeding experience in some species (Motes-Rodrigo, Labra, & Lampe, 2017). Evidence also suggests in species with polygynous mating systems, males with large song repertoires sire more offspring with greater viability (Hasselquist, 1998; Hiebert, Stoddard, & Arcese, 1989). Females mating with males that have large repertoires can therefore gain both direct fitness and indirect genetic benefits for their young (Westneat & Fox, 2010). Nevertheless, inconsistencies in conclusions concerning associations between reproductive success and song complexity highlight the need for further clarity and scrutiny in this area.

From an evolutionary perspective, our study contributes to some extent to the understanding of the life history of songbird species. Extrapair paternity frequency, however, can also vary within species. This may be caused by differences in stochastic local ecological and genetic factors (Arnold & Owens, 2002; Kokko & Mappes, 2013). These include differences in aggression of male territory holders (Moulton, Linz, & Bleier, 2013) and variations in genetic diversity within different populations (Liu et al., 2015). Within-species EPP frequency variation should therefore also be the focus of future field studies in association with song complexity across taxa as this is still not well understood. The accessibility and advancement of techniques in gathering genetic paternity data in songbirds should facilitate such research. These will help evaluate further the associations of song complexity and EPP.

In tui, a species with high EPP frequency, BS were more complex than IS, consistent with our prediction. This was reflected in more syllables per song and greater syllable diversity, in addition to longer song and trill duration and a higher trill percentage in BS. Higher BS complexity in species with high EPP frequency could function to maximize the chances of obtaining both social mates and multiple EP mates. This will need further testing, however, as BS in addition are likely to have multiple biological functions such as social mate attraction and territory tenure and defence because high song complexity is a signal assessed by both males and females (Leitão et al., 2006). Because BS are designed for long-distance signalling, they may also function to minimize social partner cuckoldry.

Higher complexity in tui BS contrasts with earlier studies in the dark-eyed junco (Titus, 1998), in which complexity was found to be higher in IS than in BS. The dark-eyed junco has a lower EPP frequency (ca. 27%) than tui (57%). Similarly, in species with lower EPP frequency such as song sparrows (ca. 24%, Hill, Akçay, Campbell, & Beecher, 2011), male–male interactive aggressive 'warbled, soft' songs are not only more complex than BS but also more variable and exhibit less stereotypy than BS (Anderson, Nowicki, & Searcy, 2007; Anderson, Searcy, Peters, & Nowicki, 2008). This suggests increases in BS song complexity are under stronger sexual selection in species with high EPP frequency. Further studies, however, are required to clarify whether the disparities in complexity between BS and IS across all taxa are associated with EPP frequency.

Higher proportions of trills were also found in tui BS than IS. Rapid trill components in songbird songs are costly to produce and are thought to be assessed by females as they are indicators of body condition and genetic quality (Ballentine, Hyman, & Nowicki, 2004; Hennin, Barker, Bradley, & Mennill, 2009). Furthermore, trill consistency is positively associated with male EPP frequency (Cramer, 2013) and trills are also thought to be important in male territory defence (Secondi, den Hartog, & ten Cate, 2002). Tui may produce more complex songs with a greater trill proportion in BS to deter potential rivals (Hill et al., 2015). This would function to minimize the risk of losing paternity via EP copulations (e.g. Reichard et al., 2011).

Conclusions

Extrapair paternity frequency varies in multiple songbird species and is correlated with the degree of song complexity. Our results suggest EP mate selection may play a role in within-song complexity evolution in songbirds. This study contributes to our understanding of variability in songbird within-song complexity between species, a phenomenon that has puzzled ecological and evolutionary researchers. Further research is required to investigate the influence of within-song complexity on the reproductive fitness of individuals in a within- and extrapair paternity framework to confirm the role of male song complexity in EPP mate selection.

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Supplementary material

Supplementary material associated with this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2017.06.026.

References

Abdi, H. (2007). Z-scores. In N. J. Salkind (Ed.), *Encyclopedia of measurement and statistics* (pp. 1057–1058). Thousand Oaks, CA: Sage Publications.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology, 26*, 32–46.

Anderson, R. C., Nowicki, S., & Searcy, W. A. (2007). Soft song in song sparrows: Response of males and females to an enigmatic signal. *Behavioral Ecology and Sociobiology, 61*, 478–484.

Anderson, R. C., Searcy, W. A., Peters, S., & Nowicki, S. (2008). Soft song in song sparrows: Acoustic structure and implications for signal function. *Ethology, 114*, 662–672.

Andersson, M. (1994). *Sexual selection*. New Jersey, NJ: Princeton University Press.

Arnold, K. E., & Owens, I. P. F. (2002). Extra-pair paternity and egg dumping in birds: Life history, parental care and the risk of retaliation. *Proceedings of the Royal Society B: Biological Sciences, 269*, 1263–1269.

Badyaev, A. V., Hill, G. E., & Weckworth, B. V. (2002). Species divergence in sexually selected traits: Increase in song elaboration is related to decrease in plumage ornamentation in finches. *Evolution, 56*, 412–419. http://dx.doi.org/10.1111/j.0014-3820.2002.tb01350.

Ballentine, B., Badyaev, A., & Hill, G. E. (2003). Changes in song complexity correspond to periods of female fertility in blue grosbeaks (*Guiraca caerulea*). *Ethology, 109*, 55–66.

Ballentine, B., Hyman, J., & Nowicki, S. (2004). Vocal performance influences female response to male bird song: An experimental test. *Behavioral Ecology, 15*, 163–168.

Barger, N., Hanson, K. L., Teffer, K., Schenker-Ahmed, N. M., & Semendeferi, K. (2014). Evidence for evolutionary specialization in human limbic structures. *Frontiers in Human Neuroscience, 8*, 277.

Birkhead, T. R. (1998). Sperm competition in birds. *Reviews of Reproduction, 3*, 123–129.

Boogert, N. J., Giraldeau, L. A., & Lefebvre, L. (2008). Song complexity correlates with learning ability in zebra finch males. *Animal Behaviour, 76*, 1735–1741.

Bowers, E. K., Forsman, A. M., Masters, B. S., Johnson, B. G. P., Johnson, L. S., Sakaluk, S. K., et al. (2015). Increased extra-pair paternity in broods of aging males and enhanced recruitment of extra-pair young in a migratory bird. *Evolution, 69*, 2533–2541.

Buchanan, K. L., & Catchpole, C. K. (2000). Song as an indicator of male parental effort in the sedge warbler. *Proceedings of the Royal Society B: Biological Sciences, 267*, 321–326.

Burnham, C. A., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (4th ed.). New York, NY: Springer.

Byers, B. E. (2007). Extrapair paternity in chestnut-sided warblers is correlated with consistent vocal performance. *Behavioral Ecology, 18*, 130–136.

Byers, B. E., & Kroodsma, D. E. (2009). Female mate choice and songbird song repertoires. *Animal Behaviour, 77*, 13–22.

Canal, D., Jovani, R., & Potti, J. (2012). Male decisions or female accessibility? Spatiotemporal patterns of extra pair paternity in a songbird. *Behavioral Ecology, 23*, 1146–1153.

Catchpole, C. K. (1980). Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour, 74*, 149–166.

Catchpole, C. K. (1983). Variation in the song of the great reed warbler *Acrocephalus arundinaceus* in relation to mate attraction and territorial defence. *Animal Behaviour, 31*, 217–1225.

Catchpole, C. K. (1986). Song repertoires and reproductive success in the great reed warbler *Acrocephalus arundinaceus*. *Behavioral Ecology and Sociobiology, 19*, 439–445.

Catchpole, C. K. (1992). Integrating playback: A wider context. In P. K. McGregor (Ed.), *Playback and Studies of Animal Communication* (pp. 35–46). Berlin, Germany: Springer Science and Business Media.

Catchpole, C. K., Leisler, B., & Dittami, J. (1986). Sexual differences in the responses of captive great reed warblers *Acrocephalus arundinaceus*, to variation in song structure and size. *Ethology, 73*, 69–77.

Catchpole, C. K., & Slater, P. J. B. (2008). *Bird song: Biological themes and variations* (2nd ed.). Cambridge, U.K.: Cambridge University Press.

Clarke, K. R., & Warwick, R. M. (1994). Similarity-based testing for community pattern: The 2-way layout with no replication. *Marine Biology, 118*, 167–176.

Craig, J. L. (1984). Wing noises, wing slots, and aggression in New Zealand honeyeaters (aves, meliphagidae). *New Zealand Journal of Zoology, 11*, 195–199.

Cramer, E. R. A. (2013). Physically challenging song traits, male quality, and reproductive success in house wrens. *PLoS ONE, 8*, e59208. http://dx.doi.org/10.1371/journal.pone.0059208.

Feßl, B., & Hoi, H. (2000). Song complexity and song structure in the moustached warbler Acrocephalus melanopogon. *Journal of Avian Biology, 31*, 144–150.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T., & Kempenaers, B. (2003). Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature, 425*, 714717.

Forstmeier, W., Kempenaers, B., Meyer, A., & Leisler, B. (2002). A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proceedings of the Royal Society B: Biological Sciences, 269*, 1479–1485.

Forstmeier, W., Nakagawa, S., Griffith, S. C., & Kempenaers, B. (2014). Female extra-pair mating: Adaptation or genetic constraint? *Trends in Ecology & Evolution, 29*, 456–464.

Garamszegi, L. Z., & Møller, A. P. (2004). Extrapair paternity and the evolution of bird song. *Behavioral Ecology, 15*, 508–519.

Gerlach, N. M., McGlothlin, J. W., Parker, P. G., & Ketterson, E. D. (2012). Reinterpreting bateman gradients: Multiple mating and selection in both sexes of a songbird species. *Behavioral Ecology, 23*, 1078–1088.

Gil, D., & Slater, P. J. B. (2000). Multiple song repertoire characteristics in the willow warbler (*Phylloscopus trochilus*): Correlations with female choice and offspring viability. *Behavioral Ecology and Sociobiology, 47*, 319–326.

Gray, E. M. (1997). Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. *Animal Behaviour, 53*, 625–639.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica, 4*, 1–9.

Hasselquist, D. (1998). Polygyny in great reed warblers: A long-term study of factors contributing to male fitness. *Ecology, 79*, 2376–2390.

Hasselquist, D., Bensch, S., & Vonschantz, T. (1995). Low frequency of extrapair paternity in the polygynous great reed warbler, *Acrocephalus arundinaceus*. *Behavioral Ecology, 6*, 27–38.

Hasselquist, D., Bensch, S., & Von Schantz, T. (1996). Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature, 381*, 229–232. http://dx.doi.org/10.1038/381229a0.

Hasselquist, D., & Sherman, P. W. (2001). Social mating systems and extrapair fertilizations in passerine birds. *Behavioral Ecology, 12*, 457–466. http://dx.doi.org/10.1093/beheco/12.4.457.

Hennin, H. L., Barker, N. K. S., Bradley, D. W., & Mennill, D. J. (2009). Bachelor and paired male rufous-and-white wrens use different singing strategies. *Behavioural Ecology and Sociobiology, 64*, 151–159.

Hiebert, S. M., Stoddard, P. K., & Arcese, P. (1989). Repertoire size, territory acquisition, and reproductive success in the song sparrow. *Animal Behaviour, 37*, 266–273.

Hill, S. D. (2011). *The vocalisation of tui (Prosthemadera novaeseelandiae)* (Masters thesis). Auckland, New Zealand: Massey University.

Hill, C. E., Akçay, C., Campbell, E., & Beecher, M. D. (2011). Extrapair paternity, song, and genetic quality in song sparrows. *Behavioral Ecology, 22*, 73–81.

Hill, S. D., Amiot, C., Ludbrook, M. R., & Ji, W. (2015). Seasonal variation in the song structure of tui (*Prosthemadera novaeseelandiae*). *New Zealand Journal of Ecology, 39*, 110–115.

Hill, S. D., & Ji, W. (2014). Categorisation of common syllable types in the complex vocalisations of tui (*Prosthemadera novaeseelandiae*). *Notornis, 61*, 54–56.

Hill, S. D., Ji, W., Parker, K. A., Amiot, C., & Wells, S. J. (2013). A comparison of vocalisations between mainland tui (*Prosthemadera novaeseelandiae novaeseelandiae*) and Chatham Island tui (*P. n. chathamensis*). *New Zealand Journal of Ecology, 37*, 214–223.

Huang, H., & Rabosky, D. L. (2015). Sex-linked genomic variation and its relationship to avian plumage dichromatism and sexual selection. *BMC Evolutionary Biology, 15*, 199. http://dx.doi.org/10.1186/s12862-015-0480-4.

Illes, A. E., Hall, M. L., & Vehrencamp, S. L. (2006). Vocal performance influences male receiver response in the banded wren. *Proceedings of the Royal Society B: Biological Sciences, 273*, 1907–1912.

Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature, 491*, 444–448.

Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., Redding, D., & Mooers, A. O. (2014). Distribution and conservation of global evolutionary distinctness in birds. *Current Biology, 24*, 1–12.

Jurisevic, M. A., & Sanderson, K. J. (1994). The vocal repertoires of six honeyeater (Meliphagidae) species from Adelaide, South Australia. *Emu, 94*, 141–148.

Kaluthota, C. D. (2016). *Sexual selection, breeding behaviour and song communication in house wrens* (Troglodytes aedon) *(Doctoral thesis)*. Lethbridge, Canada: University of Lethbridge. Retrieved from http://hdl.handle.net/10133/4559.

Kempenaers, B., Verheyen, G. R., Van Den Broeck, M., Burke, T., Van Broeckhoven, C., & Dhondt, A. (1992). Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature, 357*, 494496. http://dx.doi.org/10.1038/357494a0.

Kokko, H., & Mappes, J. (2013). Multiple mating by females is a natural outcome of a null model of mate encounters. *Entomologia Experimentalis et Applicata, 146*, 26–37.

Kroodsma, D. E. (1977). Correlates of song organisation among North American wrens. *American Naturalist, 111*, 995–1008.

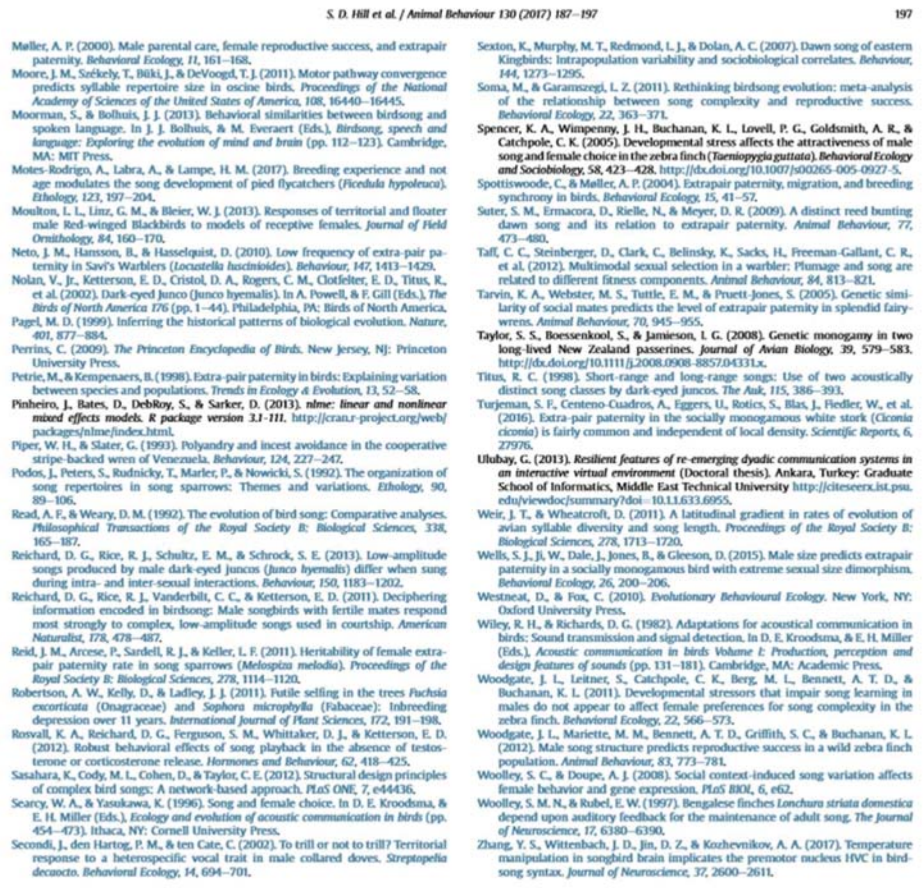
Leitão, A., ten Cate, C., & Riebel, K. (2006). Song complexity in a songbird is meaningful to both male and female receivers. *Animal Behaviour, 71*, 1289–1296.

Leniowski, K., & Wegrzyn, E. (2013). Organization, variation in time, and impacting factors in the song strophe repertoire in the Great Reed Warbler (*Acrocephalus arundinaceus*). *Ornis Fennica, 90*, 129–141.

Lifjeld, J. T., Laskemoen, T., Kleven, O., Albrecht, T., & Robertson, R. J. (2010). Sperm length variation as a predictor of extrapair paternity in passerine birds. *PLoS ONE, 5*, e13456. http://dx.doi.org/10.1371/journal.pone.0013456.

Liu, I. A., Johndrow, J. E., Abe, J., Lüpold, S., Yasukawa, K., Westneat, D. F., et al. (2015). Genetic diversity does not explain variation in extra-pair paternity in multiple populations of a songbird. *Journal of Evolutionary Biology, 28*, 1156–1169.

Marshall, R. C., Buchanan, K. L., & Catchpole, C. K. (2007). Song and female choice for extrapair copulations in the sedge warbler. *Acrocephalus schoenobaenus*. *Animal Behaviour, 73*, 629–635.



Møller, A. P. (2000). Male parental care, female reproductive success, and extrapair paternity. *Behavioral Ecology, 11*, 161–168.

Moore, J. M., Székely, T., Büki, J., & DeVoogd, T. J. (2011). Motor pathway convergence predicts syllable repertoire size in oscine birds. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 16440–16445.

Moorman, S., & Bolhuis, J. J. (2013). Behavioral similarities between birdsong and spoken language. In J. J. Bolhuis, & M. Everaert (Eds.), *Birdsong, speech and language: Exploring the evolution of mind and brain* (pp. 112–123). Cambridge, MA: MIT Press.

Motes-Rodrigo, A., Labra, A., & Lampe, H. M. (2017). Breeding experience and not age modulates the song development of pied flycatchers (*Ficedula hypoleuca*). *Ethology, 123*, 197–204.

Moulton, L. L., Linz, G. M., & Bleier, W. J. (2013). Responses of territorial and floater male Red-winged Blackbirds to models of receptive females. *Journal of Field Ornithology, 84*, 160–170.

Neto, J. M., Hansson, B., & Hasselquist, D. (2010). Low frequency of extra-pair paternity in Savi's Warblers (*Locustella luscinioides*). *Behaviour, 147*, 1413–1429.

Nolan, V., Jr., Ketterson, E. D., Cristol, D. A., Rogers, C. M., Clotfelter, E. D., Titus, R., et al. (2002). Dark-eyed Junco (Junco hyemalis). In A. Powell, & F. Gill (Eds.), *The Birds of North America 176* (pp. 1–44). Philadelphia, PA: Birds of North America.

Pagel, M. D. (1999). Inferring the historical patterns of biological evolution. *Nature, 401*, 877–884.

Perrins, C. (2009). *The Princeton Encyclopedia of Birds*. New Jersey, NJ: Princeton University Press.

Petrie, M., & Kempenaers, B. (1998). Extra-pair paternity in birds: Explaining variation between species and populations. *Trends in Ecology & Evolution, 13*, 52–58.

Pinheiro, J., Bates, D., DebRoy, S., & Sarker, D. (2013). *nlme: linear and nonlinear mixed effects models. R package version 3.1-111.* http://cran.r-project.org/web/packages/nlme/index.html.

Piper, W. H., & Slater, G. (1993). Polyandry and incest avoidance in the cooperative stripe-backed wren of Venezuela. *Behaviour, 124*, 227–247.

Podos, J., Peters, S., Rudnicky, T., Marler, P., & Nowicki, S. (1992). The organization of song repertoires in song sparrows: Themes and variations. *Ethology, 90*, 89–106.

Read, A. F., & Weary, D. M. (1992). The evolution of bird song: Comparative analyses. *Philosophical Transactions of the Royal Society B: Biological Sciences, 338*, 165–187.

Reichard, D. G., Rice, R. J., Schultz, E. M., & Schrock, S. E. (2013). Low-amplitude songs produced by male dark-eyed juncos (*Junco hyemalis*) differ when sung during intra- and inter-sexual interactions. *Behaviour, 150*, 1183–1202.

Reichard, D. G., Rice, R. J., Vanderbilt, C. C., & Ketterson, E. D. (2011). Deciphering information encoded in birdsong: Male songbirds with fertile mates respond most strongly to complex, low-amplitude songs used in courtship. *American Naturalist, 178*, 478–487.

Reid, J. M., Arcese, P., Sardell, R. J., & Keller, L. F. (2011). Heritability of female extra-pair paternity rate in song sparrows (*Melospiza melodia*). *Proceedings of the Royal Society B: Biological Sciences, 278*, 1114–1120.

Robertson, A. W., Kelly, D., & Ladley, J. J. (2011). Futile selfing in the trees *Fuchsia excorticata* (Onagraceae) and *Sophora microphylla* (Fabaceae): Inbreeding depression over 11 years. *International Journal of Plant Sciences, 172*, 191–198.

Rosvall, K. A., Reichard, D. G., Ferguson, S. M., Whittaker, D. J., & Ketterson, E. D. (2012). Robust behavioral effects of song playback in the absence of testosterone or corticosterone release. *Hormones and Behaviour, 62*, 418–425.

Sasahara, K., Cody, M. L., Cohen, D., & Taylor, C. E. (2012). Structural design principles of complex bird songs: A network-based approach. *PLoS ONE, 7*, e44436.

Searcy, W. A., & Yasukawa, K. (1996). Song and female choice. In D. E. Kroodsma, & E. H. Miller (Eds.), *Ecology and evolution of acoustic communication in birds* (pp. 454–473). Ithaca, NY: Cornell University Press.

Secondi, J., den Hartog, P. M., & ten Cate, C. (2002). To trill or not to trill? Territorial response to a heterospecific vocal trait in male collared doves. *Streptopelia decaocto. Behavioral Ecology, 14*, 694–701.

Sexton, K., Murphy, M. T., Redmond, L. J., & Dolan, A. C. (2007). Dawn song of eastern Kingbirds: Intrapopulation variability and sociobiological correlates. *Behaviour, 144*, 1273–1295.

Soma, M., & Garamszegi, L. Z. (2011). Rethinking birdsong evolution: meta-analysis of the relationship between song complexity and reproductive success. *Behavioral Ecology, 22*, 363–371.

Spencer, K. A., Wimpenny, J. H., Buchanan, K. L., Lovell, P. G., Goldsmith, A. R., & Catchpole, C. K. (2005). Developmental stress affects the attractiveness of male song and female choice in the zebra finch (*Taeniopygia guttata*). *Behavioral Ecology and Sociobiology, 58*, 423–428. http://dx.doi.org/10.1007/s00265-005-0927-5.

Spottiswoode, C., & Møller, A. P. (2004). Extrapair paternity, migration, and breeding synchrony in birds. *Behavioral Ecology, 15*, 41–57.

Suter, S. M., Ermacora, D., Rielle, N., & Meyer, D. R. (2009). A distinct reed bunting dawn song and its relation to extrapair paternity. *Animal Behaviour, 77*, 473–480.

Taff, C. C., Steinberger, D., Clark, C., Belinsky, K., Sacks, H., Freeman-Gallant, C. R., et al. (2012). Multimodal sexual selection in a warbler: Plumage and song are related to different fitness components. *Animal Behaviour, 84*, 813–821.

Tarvin, K. A., Webster, M. S., Tuttle, E. M., & Pruett-Jones, S. (2005). Genetic similarity of social mates predicts the level of extrapair paternity in splendid fairy-wrens. *Animal Behaviour, 70*, 945–955.

Taylor, S. S., Boessenkool, S., & Jamieson, I. G. (2008). Genetic monogamy in two long-lived New Zealand passerines. *Journal of Avian Biology, 39*, 579–583. http://dx.doi.org/10.1111/j.2008.0908-8857.04331.x.

Titus, R. C. (1998). Short-range and long-range songs: Use of two acoustically distinct song classes by dark-eyed juncos. *The Auk, 115*, 386–393.

Turjeman, S. F., Centeno-Cuadros, A., Eggers, U., Rotics, S., Blas, J., Fiedler, W., et al. (2016). Extra-pair paternity in the socially monogamous white stork (*Ciconia ciconia*) is fairly common and independent of local density. *Scientific Reports, 6*, 27976.

Ulubay, G. (2013). *Resilient features of re-emerging dyadic communication systems in an interactive virtual environment* (Doctoral thesis). Ankara, Turkey: Graduate School of Informatics, Middle East Technical University http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.633.6955.

Weir, J. T., & Wheatcroft, D. (2011). A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Proceedings of the Royal Society B: Biological Sciences, 278*, 1713–1720.

Wells, S. J., Ji, W., Dale, J., Jones, B., & Gleeson, D. (2015). Male size predicts extrapair paternity in a socially monogamous bird with extreme sexual size dimorphism. *Behavioral Ecology, 26*, 200–206.

Westneat, D., & Fox, C. (2010). *Evolutionary Behavioural Ecology*. New York, NY: Oxford University Press.

Wiley, R. H., & Richards, D. G. (1982). Adaptations for acoustical communication in birds: Sound transmission and signal detection. In D. E. Kroodsma, & E. H. Miller (Eds.), *Acoustic communication in birds Volume I: Production, perception and design features of sounds* (pp. 131–181). Cambridge, MA: Academic Press.

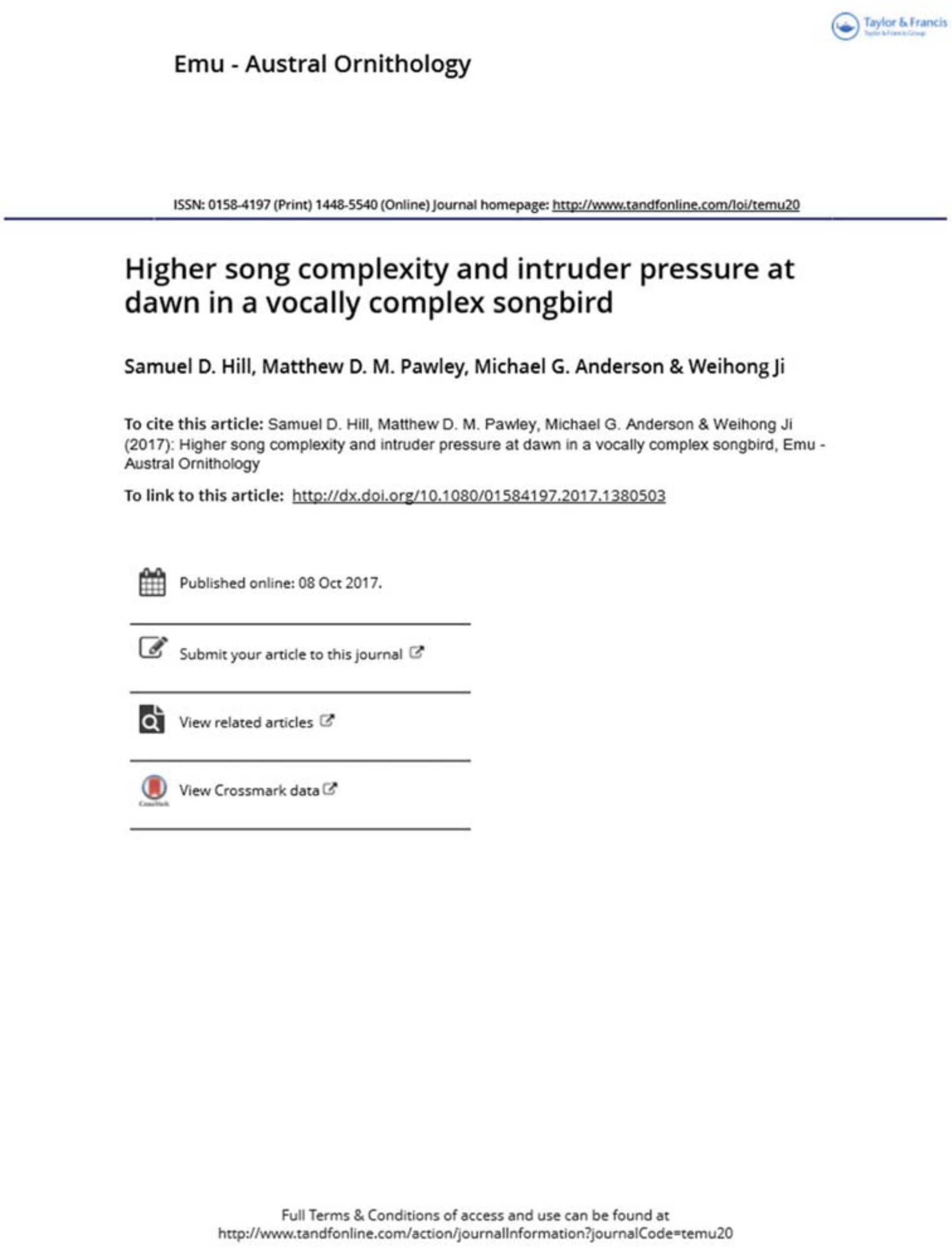
Woodgate, J. L., Leitner, S., Catchpole, C. K., Berg, M. L., Bennett, A. T. D., & Buchanan, K. L. (2011). Developmental stressors that impair song learning in males do not appear to affect female preferences for song complexity in the zebra finch. *Behavioral Ecology, 22*, 566–573.

Woodgate, J. L., Mariette, M. M., Bennett, A. T. D., Griffith, S. C., & Buchanan, K. L. (2012). Male song structure predicts reproductive success in a wild zebra finch population. *Animal Behaviour, 83*, 773–781.

Woolley, S. C., & Doupe, A. J. (2008). Social context-induced song variation affects female behavior and gene expression. *PLoS BIOL, 6*, e62.

Woolley, S. M. N., & Rubel, E. W. (1997). Bengalese finches *Lonchura striata domestica* depend upon auditory feedback for the maintenance of adult song. *The Journal of Neuroscience, 17*, 6380–6390.

Zhang, Y. S., Wittenbach, J. D., Jin, D. Z., & Kozhevnikov, A. A. (2017). Temperature manipulation in songbird brain implicates the premotor nucleus HVC in birdsong syntax. *Journal of Neuroscience, 37*, 2600–2611.



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Higher song complexity and intruder pressure at dawn in a vocally complex songbird

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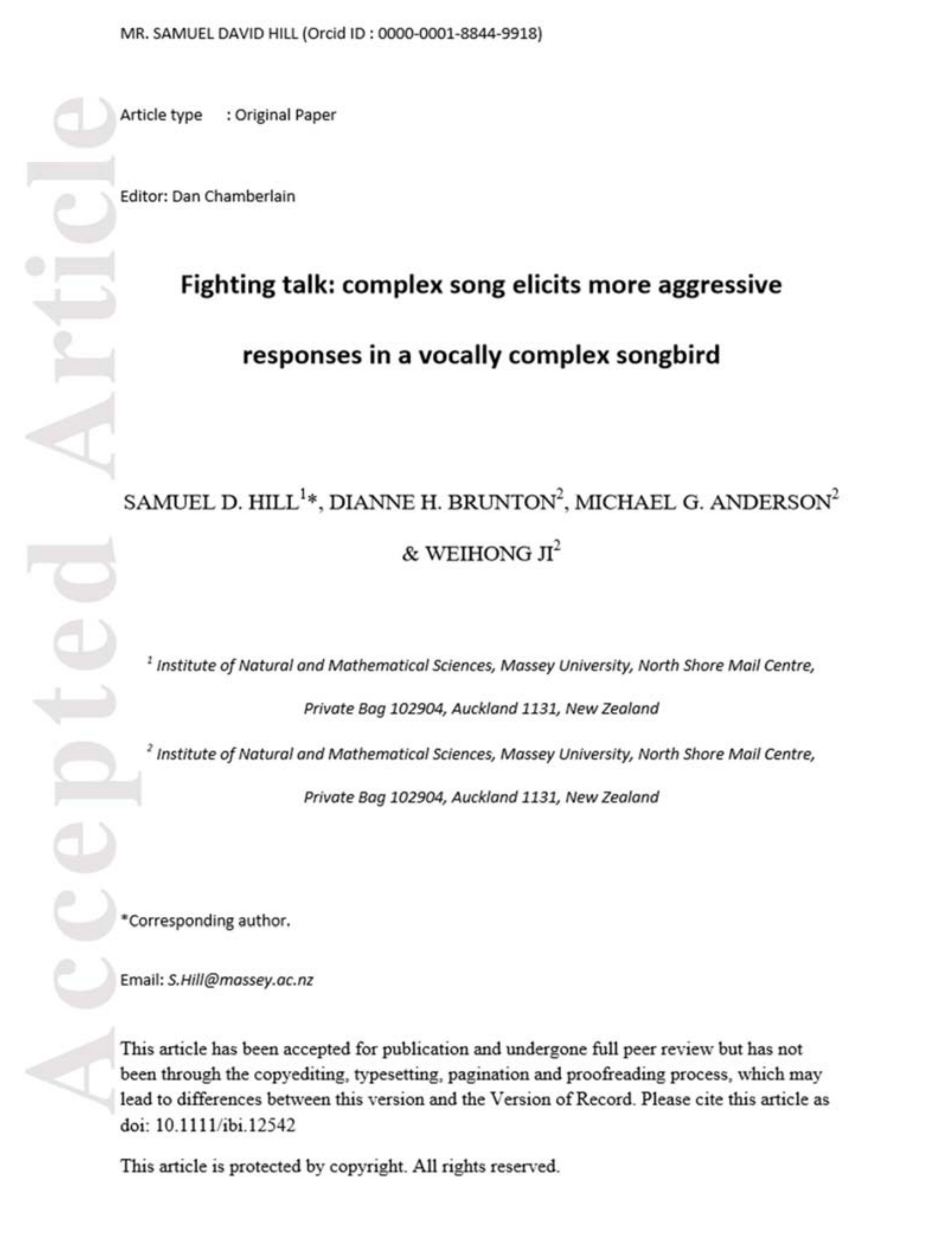

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Fighting talk: complex song elicits more aggressive responses in a vocally complex songbird

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Song complexity in many songbirds is a trait subject to sexual selection. It is often associated with male territorial defence. Empirical studies testing differential male responses to rival song in vocally complex songbirds have, however, been scarce. We conducted playback experiments of the endemic New Zealand Tui *Prosthemadera novaeseelandiae* to test the aggressive response of territorial male Tui to rival songs with differing complexity levels. Overall, complex songs evoked significantly stronger responses from territorial males than simple songs. Following playback of complex songs, focal males approached the playback more closely and rapidly, and responded with songs of higher complexity than to playback of simple songs. This suggests males could both distinguish between different levels of complexity within the Tui repertoire, and perceive a more complex song as a greater territorial threat. Our study is one of the first to demonstrate strong aggressive responses to increased levels of song complexity in a songbird species with highly complex vocalisations.

Keywords: playback, rival, song complexity, territoriality.

Bird song is a communication signal with two key functions: mate attraction, and territory defence (Catchpole & Slater 2008, Brunner & Pasinelli 2010, Leedale *et al.* 2015, Moiron *et al.* 2015, Monbureau *et al.* 2015). Song production may provide advantages to the singer on both fronts, termed the 'dual function hypothesis' (Catchpole & Slater 2008). This becomes particularly important during the female fertile period (Greig-Smith 1982) when competition for breeding partners and resources is at its peak (Hjernquist *et al.* 2008; Pryke 2013).

Evidence indicates complex songs evolved due at least partly to sexual selection for such high-performance vocalisations (Catchpole 1980). Nonetheless, levels of song complexity (e.g. the number of syllables contained within a song) vary greatly across songbird taxa. This is partly due to interspecific variations in song structure (DeVoogd *et al.* 1993). The California Thrasher *Toxostoma* *redivivum* and Winter Wren *Troglodytes hiemalis,* for example, produce complex songs containing many different syllables (Kroodsma 1981, Cody *et al.* 2016). On the other hand, the White-crowned Sparrow *Zonotrichia leucophrys*, Zebra Finch *Taeniopygia guttata* and Common Chiffchaff *Phylloscopus collybita* have merely a few similar syllables within their repertoire (Marler & Tamura 1962, Zann 1996, Jaška *et al.* 2015). High songbird song complexity can be both energetically costly in terms of field metabolic rate (Garamszegi *et al.* 2006) and sexually selected (Schmidt *et al.* 2013). Song complexity may be an honest indicator of various aspects of male quality (Spencer *et al.* 2003; Cramer 2013). These factors include genetic diversity (Marshall *et al.* 2003), developmental stress levels (Spencer *et al.* 2003; Nowicki & Searcy 2004) and parasite load (Hamilton & Zuk 1982).

Song structure may be shaped by the forces of both intra- and intersexual selection (Catchpole & Slater 2008). Levels of song complexity can even vary between individuals of the same species (Feßl & Hoi 2000). Song complexity may be used by males to assess the quality of potential territorial rivals in vocally complex species (Mountjoy & Lemon 1991). Moreover, there is evidence that song complexity plays an important role in territory acquisition and defence (Beecher & Brenowitz 2005). Furthermore, males of some species, such as Red-winged Blackbirds *Agelaius phoeniceus*, de-emphasise their song repertoires in the presence of rival males (West *et al.* 1981, Searcy & Yasukawa 1990), which possibly functions to avoid costly physical conflict. However, this might depend upon the age and fitness of the receiver.

Song complexity has also been found to correlate with several reproductive success metrics. For instance, increased parental care (e.g. in Sedge Warblers *Acrocephalus schoenobaenus*, Buchanan & Catchpole 2000), initial mating success (e.g. Song Sparrows *Melospiza melodia*, Reid *et al.* 2004) and lifetime reproductive success through territory tenure (e.g. Song Sparrows, Potvin *et al.* 2015). Furthermore, males with high song complexity pair earlier (e.g. Common Starlings *Sturnus vulgaris*, Mountjoy & Lemon 1996) and are more successful in extra-pair breeding (e.g. Great Reed Warblers *Acrocephalus arundinaceus*, Hasselquist *et al.* 1996; Leisler *et al.* 2000).

Playback experiments under natural conditions can be powerful tools to investigate bird song variation, elucidate the rudimentary functions of song and examine the roles of different aspects of song structure (Catchpole *et al.* 1984; Kroodsma & Byers 1991; Kipper *et al.* 2015). Playbacks combine both the precision and accuracy of laboratory conditions with the unknown, uncontrollable, natural conditions of the field (Falls 1992).

The Tui *Prosthemadera novaeseelandiae* is an endemic New Zealand songbird in the Family Meliphagidae. Both sexes sing, although females less frequently than males (Bergquist 1989). Tui produce long-range, territorial broadcast songs that are generally long and complex in nature (Hill *et al.* 2015). They will also produce shorter and simpler broadcast songs in addition to alarm and distress calls. These two types of broadcast song may have different functions, but this is as yet unclear. Males can produce at least 56 different syllables within a single song (Hill *et al.* 2015). This large syllable repertoire results in a large and diverse song repertoire. Indeed, male Tui songs are rarely repeated as exact copies (Hill 2011) and within-individual song complexity varies (Hill *et al.* 2015). Some syllables and song strophes are nevertheless shared between neighbouring individuals (Hill & Ji 2013). While the functions of Tui song remain largely obscure, they may be used in mate attraction, dominance hierarchy resolution (Bergquist & Craig 1988, Bergquist 1989, Hill *et al.* 2015) and territoriality (Hill *et al.* 2013). Tui also have high extra-pair paternity (57%, Wells *et al.* 2015). This suite of behavioural traits makes Tui an excellent model to investigate territorial male responses to differing song complexity levels.

Our aim was to investigate breeding male Tui responses to simple and complex songs from intruding male Tui. This was tested using playback experiments to gain an understanding of the function of song complexity in the context of Tui territoriality. As songbird song complexity plays a role in territory tenure, and female extra-pair mate selection (Potvin *et al.* 2015), intruding males with more complex songs are likely to be perceived as stronger competitors by territorial males. Theoretically, this would be particularly true in species with high extra-pair paternity who risk suffering cuckoldry in addition to losing territory. Therefore, we predicted that more complex songs of intruding male Tui would indicate a greater challenge to territorial males, and thus would elicit stronger, more aggressive responses than a simpler song.

METHODS

Locations

We conducted playback experiments in 12 Tui territories in Ecology Bush, Tawharanui Regional Park, 90 km north of central Auckland, New Zealand (36°22'S, 174°50'E). Tawharanui is a 588-ha predator-fenced mainland island on the Tawharanui Peninsula (Supplementary Material 1). The park consists of grazed pasture and coastal native forest remnants where Tui are abundant. Each playback location was conducted within a territory of a focal male.

The distance between adjacent playback territories was approximately 200 m (Supplementary Material 1). To test whether stimuli from a playback speaker (SAST PT06 Loudspeaker, Shenzhen SAST Electronics Co. Ltd, Shenzhen, China) could be detected by a non-target Tui 200 m from the sound source, we recorded a randomly-selected complex song stimulus (see below for more details on the stimuli) from 200 m away (at 80 decibels relative to a carrier level [dBC] from 1 m, fast setting, measured using a sound level meter, Digitech QM-1589) using a long-range microphone that had a frequency response range of 40 Hz to 20 kHz (Sennheiser ME67, Sennheiser, Old Lyme, CT) attached to a Marantz PMD620 digital recorder (Marantz, Kanagawa, Japan). The stimulus, however, was undetectable on the spectrogram produced by Raven Pro software (1.4 Beta Version, Cornell Lab of Ornithology, Ithaca, NY, USA). Nevertheless, we conducted a playback of this recording to Tui 20 m away from the speaker (at 80 dBC), which evoked no response. Therefore 200 m was used as the distance between playback territories to minimise the chance of Tui from other playback territories detecting and habituating to the playback stimuli.

Tui are especially territorial in the breeding season and males sing regularly on specific perches within their territories (Bergquist & Craig 1988). Ringed Tui stayed within their territories throughout the duration of our playback experiments. We were therefore confident that for unringed males the same bird was at each territory on subsequent days. We identified Tui territory boundaries by watching flights and movements of Tui. The presence of a territorial male Tui does not necessarily equate to the territory holder having a mate or a nest.

Of the 12 territories, four of the territorial male Tui were ringed and eight unringed. One was ringed in 2011 and three in 2013. The breeding stages of two males were confirmed, one nest building and the other post-fledging. No other focal males had active nests that were found and were most likely in-between broods because the playbacks were conducted in mid-breeding season (on three separate days: 11, 14 and 18 December 2014).

Stimuli

The stimuli used for the playbacks were: 1) complex broadcast male Tui song; 2) simple broadcast Tui song; and, 3) control heterospecific song (of a male Grey Warbler *Gerygone igata*). Grey Warbler song was selected as a control as they are a non-related songbird commonly occurring in the same location and habitat, and can be regularly heard. Variables used to measure within-song complexity of stimuli were: 1) number of syllables (the total number of syllables within each song, Boogert *et al.* 2008), where a syllable is defined as either a continuous tracing on the spectrogram, or a tight group of identical sound units (we considered a syllable as such, if it was separated from other syllables by a visible temporal interval ≥ 90 ms); 2) syllable diversity (number of *different* syllables within each song, Garamszegi & Møller 2003, Boogert *et al.* 2008), where different syllables were identified and distinguished by their duration, spectral structure, and bandwidth (the difference between minimum and maximum frequencies); 3) syllable transitions (the number of times the bird switches from one syllable type to another, Sasahara *et al.* 2012); and, 4) song duration (length of time from the beginning of the first syllable to the end of the terminal syllable of each chosen song, Boogert *et al.* 2008; Supplementary Material 2). Tui songs were assigned to one of two categories: simple or complex based on these four variables. Within the complex Tui playback songs, the number of syllables, syllable diversity, syllable transitions, and song duration were all higher than within the simple Tui playback songs (Supplementary Material 3). In total, there were six different songs within each stimulus type category (complex, simple, and control; Fig. 1) as per the recommendations in Kroodsma (1990), but each Tui only heard one song per category (i.e. one complex, one simple and one control song, i.e. three songs heard per Tui). The mean ± standard error syllable diversity (23 ± 2) within the complex Tui song stimuli was greater than the mean of the Tawharanui Tui population (19 ± 9, Hill et al. 2013).

All Tui song stimuli were recorded from a single territorial male (ID code: YG-YM, 123 g). The stimuli were recorded near Jones Bay, approximately 1.5 km from the nearest focal Tui territory, so were unlikely to have been familiar to each focal male at each playback territory. The Grey Warbler songs used as controls were recorded from the park approximately 1.5 km from the nearest focal Tui territory, so were also unlikely to have been familiar to each focal male. All recordings written as wav files were made from the previous breeding season (at a sampling rate of 44.1 kHz, with a resolution of 24 bits). This was conducted using the long-range directional microphone attached to the digital recorder, both described above.

The recordings were edited and stimuli produced using Raven Pro (1.4 Beta Version). The sound level meter was used to ensure the sound was at the same volume for each stimulus (80 dBC at 1 m, fast setting). This represented natural singing volume as measured by a sound level meter. Each stimulus was a recording with a good signal-to-noise ratio. One different song from the source male was used for every different stimulus. Every stimulus had the same ratio of signal (i.e. song bouts) to silence over the entirety of the three minute (90 second song, 90 second silence) at intervals to simulate natural song as best as possible. Each focal male therefore was exposed to the same amount of stimuli, whether complex, simple, or control song.

Playback experiment

For the playback experiment, the position of the speaker was generally on the periphery of each focal bird's territory. This simulated a commonly observed type of territory invasion in Tui, where a male Tui will approach, loiter and sing within a safe distance from the territory owner. Prior to the playback experiments, we set out flagging tape at measured distances (2, 5, 10, 15 m) from the playback speaker in order to aid estimation of distances. We also marked speaker location so the experiment could be replicated on subsequent days (as per Searcy *et al.* 1997). The speaker was placed approximately 2 m above the ground. We waited until the territorial male was present in its regular perching area before commencing the experiment. Typically, the male would arrive in less than five minutes, although occasionally the male was already on a known singing perch.

The directional speaker was always oriented towards the birds' regular singing perch within each territory to minimise the chances of disturbing birds on neighbouring territories. We placed the speaker a minimum of 20 m from each bird's regular singing perch before initiating playback. From observations, when rival Tui intrude onto territories, they will either arrive and sing on a peripheral perch, directly approach the territory holder on its singing perch, or fly through the territory. Performing playbacks at this distance generally stimulated the former, whilst minimising human disturbance of the focal bird. Observers stood 9 m from the speaker, therefore 29 m from each bird's regular singing perch.

Songs were played back as wav files from an Emachines E443 laptop computer (Emachines, Irvine, California, USA). The laptop was attached to the speaker by a 9 m cable. The speaker had a frequency response of 200 Hz–13,000 Hz (± 1 dB), similar to natural Tui signals.

The playback procedure consisted of a three minute pre-stimulus observation period to confirm focal males were exhibiting normal behaviour (e.g. perching or foraging) and that they were not stimulated by conspecific song or already engaged in territorial confrontation. This was followed by a three minute period of randomised stimulus treatment (complex Tui song, simple Tui song, or control song), during which eight response variables were recorded (latency of first vocal response, latency of first flight or movement, distance from speaker after 30 seconds, distance from speaker after two minutes, closest distance to speaker, total number of flights towards/over/in response to stimuli, length of time within 2 m of speaker and total number of songs; Table 1). A three minute period of post-stimulus observation followed, during which five of the above-mentioned eight response variables (latency of first vocal response, latency of first flight or movement, distance from the speaker after 30 seconds, distance from the speaker after two minutes) were recorded from the focal male. Songs were shuffled and each song assigned a number. There were six songs within each of the three stimulus type categories (*n* = 18 songs), so each song was assigned a number 1-18. The 'Random Number Generator' app on an iPhone® (Apple, Cupertino, CA) was used to determine which song was to be played to each male (only one song per category was played to each Tui). We ensured that three different stimulus types (complex, simple, and control) were played at each territory across the three playback days. On day two, a random number was generated for each territory as previously, but if it was a song that had already been played within that territory on day one, another random number was generated to correspond with an unfamiliar song stimulus. We did the same on day three to ensure songs from all three stimulus types had been played at each territory over the three days without any being repeated.

During the playback experiment, we used three observers to record the response of the focal bird (two of whom were unaware of the hypothesis, i.e. blind). A fourth person acted as an observer to track the movement of the focal male should it fly out of sight of the three main observers. The observations from all three observers had to agree for that behavioural measure to be included. We had unanimous between-observer agreement, therefore no behavioural measures were excluded. Vocal responses of the focal males were simultaneously recorded using the long-range directional microphone attached to the digital recorder during and post-stimulus.

The intervals of three and four days between playback sessions respectively to each focal male minimised the chances of habituation to song stimuli. The playbacks were conducted between 0600 and 0930 during dry and still weather. Rain and wind gusts occurred on one occasion during the playback experiments but cleared within approximately 12 minutes. The playback was paused and continued once the weather was dry and still again.

For unringed Tui, it was possible to differentiate males from females, due to sexual dimorphism. Males are significantly larger on average than females (Wells *et al.* 2015). The sex and the number of individuals responding were noted during each playback session.

Statistical analysis

A principal component analysis (PCA) was used to combine the eight correlated measures of response into fewer orthogonal measures. The complexity measures of vocal responses were analysed separately (see below). Three values per individual, per variable, were entered into the model as independent data points (complex, simple, and control). Data were non-normally distributed and so were normalised using log transformations to ensure even standard deviations across the variables. PCA is useful in playback experiment analyses, as it makes the interpretation of responses to playback easier and avoids the statistical issues concerned with correlated measures. Post-hoc tests were run to determine significant differences between the different combinations of related groups. Firstly, a Friedman’s test was used to detect differences in the strength of responses between treatments (i.e. stimuli) across multiple test attempts. Friedman’s test incorporates variation in individual males, i.e. it accounts for repeated measures. Where Friedman’s tests were significant, post-hoc Wilcoxon matched-pairs signed rank tests with Bonferroni correction were run on pairs of variables in order to identify significant differences in the aggressiveness of response to the stimuli for each of the response variables (one at a time). Wilcoxon tests were conducted on the different combinations of groups (complex, simple and control song). We also compared the resulting PCA scores using Wilcoxon matched-pairs signed rank tests between treatments.

To further test the strength of responses from the three stimuli, the raw response variables were examined by analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA; Clarke 1993, Anderson 2001) both of which perform multiple permutations of the data, thereby increasing the robustness of the statistical analysis augmenting the PCA, Friedman's and Wilcoxon tests. ANOSIM is a non-parametric multivariate test based on Euclidean distance, using the rank order of dissimilarity values to test whether the strength of responses (of the eight main response variables) differed significantly between the three stimuli. A one-way PERMANOVA based on Euclidean distance was also used to test whether the strength of responses differed significantly between the three stimuli types. PERMANOVA compares groups of objects and tests the null hypothesis that group centroids and dispersion are the same for all groups, based on the distance measure. PERMANOVA was run on matrices of Euclidean dissimilarity calculated from raw response variables that were standardised to Z-scores (i.e. normalisation). It partitioned sources of variation with methodology analogous to ANOVA and used unrestricted permutations of raw data to test for statistical significance in the data (Anderson 2001, Olabarria *et al.* 2010). Multivariate PERMANOVA is highly robust to correlations and heterogeneous variances (Anderson & Walsh 2013).

Complexity measures of vocal responses

A single experienced observer used Raven Pro to collect four measures of within-song complexity from the vocal responses to stimuli: number of syllables per song, syllable diversity per song, syllable transitions per song, and song duration (as described in the 'stimuli' subsection). Some songs after being analysed were re-evaluated to maximise consistency. Not all focal males responded vocally to the song stimuli during the playback experiment. Song complexity therefore was not included in the analysis alongside the other eight response variables because song complexity can only be measured when vocalisations are produced. Instead, we used one-way ANOSIM and PERMANOVA tests to compare complexity of any vocal responses from focal males in response to the different stimuli. A mean of three response songs were analysed for every focal male that responded with songs.

The ANOSIM and PERMANOVA were performed using PAST software version 2.17b (Hammer *et al.* 2001). The ANOSIM and PERMANOVA tests were conducted with 9999 permutations, and with the Bonferroni correction for multiple comparisons. All other statistical procedures were conducted using SPSS (version 20.0, SPSS, Chicago, IL, USA).

RESULTS

There were 36 playback sessions conducted in total (24 Tui song playbacks and 12 control song playbacks). In 19 of the 24 Tui song playback sessions (79% of the total number of Tui song playbacks), only males responded. Females responded, physically but non-vocally, to complex song on four occasions, and simple song on one occasion. On these occasions, females approached the focal male non-aggressively and perched alongside the focal male. More than one male responded in 14 Tui song playback sessions (58%), and in nine of these 14 sessions (64%), the responses were exclusively to the complex song stimuli. In five of these 14 sessions (36%), males responded to both complex *and* simple stimuli. It is possible that other individuals aside from the focal bird responded to both the song stimuli and subsequent vocalisations of the focal male. Only the focal male's response measurements, however, were included in the statistical analysis.

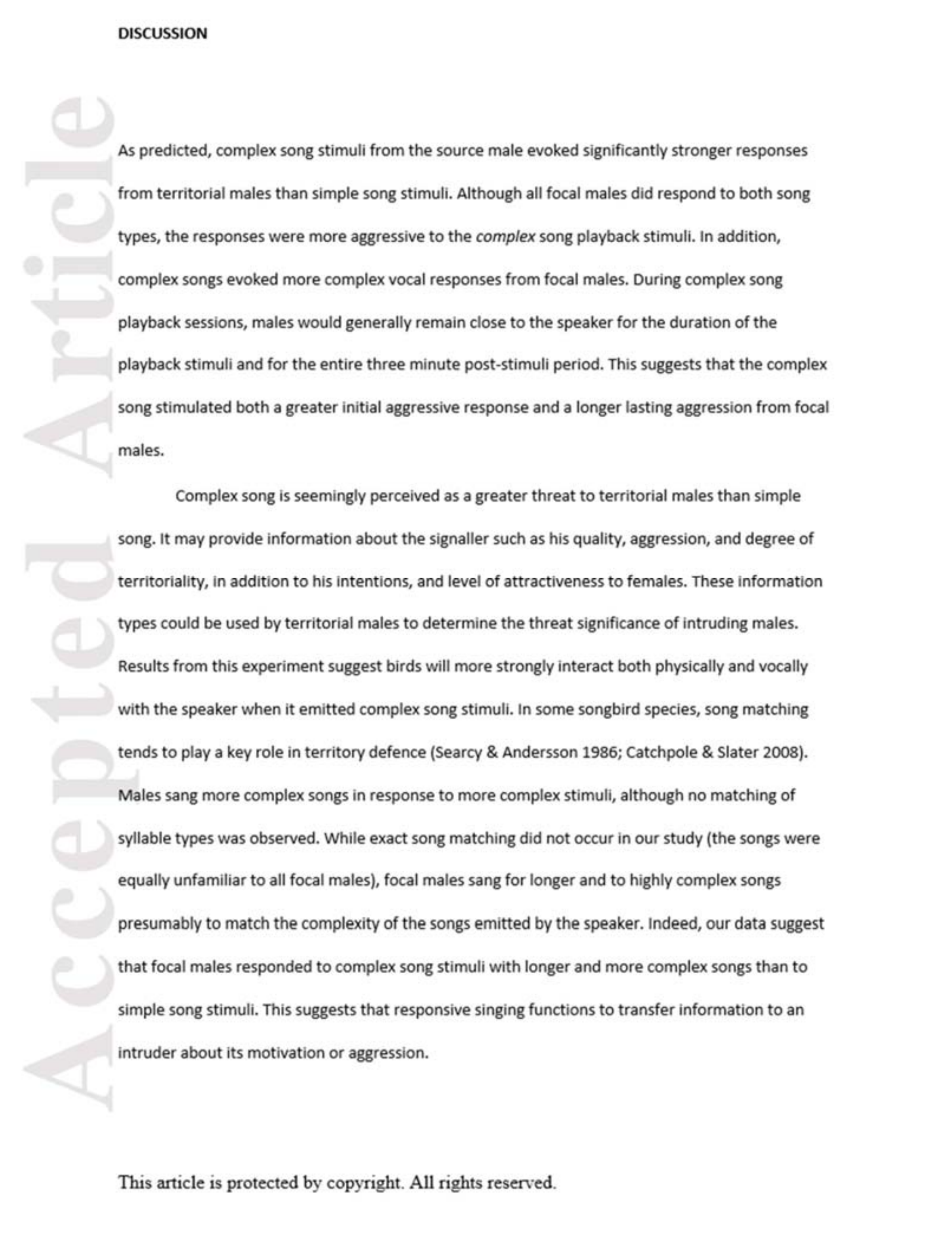
The PCA for the response variables showed that PC1 explained 71.69% of the variance between the three groups, whereas PC2 explained 12.37% (Table 2). A Friedman's test on the raw response variable values for each individual revealed that there were significant differences in the strength of response across the three different stimuli ($\chi^2_2 = 19.5$, $P < 0.001$), i.e. this allowed us to reject the null hypothesis that there were no differences in the strength of response for all eight main variables to all three stimuli. There were also significant differences in PC1 scores between treatments (Table 3). Coupled with the scatterplot (Fig. 2), this also suggests there were differences in the strength of response of the focal birds to the three different stimuli.

The PCA eigenvalues suggested all variables except length of time within 2 m of the speaker were correlated (Table 2). A post-hoc Wilcoxon matched-pairs signed ranks test (with Bonferroni correction) was conducted on the variable that contributed the most to the variation between the groups on the first PC. This variable essentially represented these seven variables measuring the aggressiveness of response. The variable contributing the highest variance on PC2 was also selected for further analysis (total number songs produced during and post-stimulus), even though PC2 contributed little to the variance between the groups. The two variables that contributed the most to the variation between the different stimuli on PC1 and PC2 respectively were: the closest distance to speaker, and the total number of songs produced during/post-stimuli (total of six minutes). Responses of all variables to the complex song stimuli were stronger than simple and control song stimuli, suggesting complex song evoked more aggressive responses from the focal males within their territory (Table 3).

One-way ANOSIM and PERMANOVA tests showed a significantly stronger response of territorial males to complex than simple song (ANOSIM $N = 12$, $R = 0.385$, $P < 0.001$; PERMANOVA *Pseudo* $F_{3, 12} = 7.325$, $P = 0.002$) and control song (ANOSIM $R = 0.995$, $P < 0.001$; PERMANOVA *Pseudo* $F_{3, 12} = 83.35$, $P < 0.001$) suggesting complex song stimuli evoked more aggressive responses from focal males than simple or control song.

Complexity of vocal responses

The complexity values of vocal responses were averaged for each individual before conducting multivariate analyses. Males had a significantly higher response to complex song than to simple song for all four song complexity variables (ANOSIM $N = 6, 8$, $R = 0.5092$, $P = 0.005$; PERMANOVA *Pseudo* $F_{9, 14} = 11.44$, $P = 0.005$; Table 4, Fig. 3). There were no song responses to any of the control stimuli.



DISCUSSION

As predicted, complex song stimuli from the source male evoked significantly stronger responses from territorial males than simple song stimuli. Although all focal males did respond to both song types, the responses were more aggressive to the *complex* song playback stimuli. In addition, complex songs evoked more complex vocal responses from focal males. During complex song playback sessions, males would generally remain close to the speaker for the duration of the playback stimuli and for the entire three minute post-stimuli period. This suggests that the complex song stimulated both a greater initial aggressive response and a longer lasting aggression from focal males.

Complex song is seemingly perceived as a greater threat to territorial males than simple song. It may provide information about the signaller such as his quality, aggression, and degree of territoriality, in addition to his intentions, and level of attractiveness to females. These information types could be used by territorial males to determine the threat significance of intruding males. Results from this experiment suggest birds will more strongly interact both physically and vocally with the speaker when it emitted complex song stimuli. In some songbird species, song matching tends to play a key role in territory defence (Searcy & Andersson 1986; Catchpole & Slater 2008). Males sang more complex songs in response to more complex stimuli, although no matching of syllable types was observed. While exact song matching did not occur in our study (the songs were equally unfamiliar to all focal males), focal males sang for longer and to highly complex songs presumably to match the complexity of the songs emitted by the speaker. Indeed, our data suggest that focal males responded to complex song stimuli with longer and more complex songs than to simple song stimuli. This suggests that responsive singing functions to transfer information to an intruder about its motivation or aggression.

Singing more complex songs has been linked with males obtaining more extra-pair fertilisations in other songbird species (Hasselquist *et al.* 1996). In Tui, a species with high extra-pair paternity frequency (57%, Wells *et al.* 2015), complex song is likely perceived as a threat to territorial males due to the possibility of extra-pair copulations of the intruder with a local female. There would be a steep drop in a territory holder's fitness if his partner increased extra-pair copulations (Kokko 1999; Eliassen & Jørgensen 2014), hence rigorous territoriality is often required to mitigate this risk. It is unclear at present whether Tui produce more complex songs in the presence of females. However, this could partly explain the more aggressive responses to males of complex song, which may indicate the presence of a female.

The playback of complex song stimuli during our trials induced a greater extent of physical aggression between responding males. This gives further support to the role that complex songs play in territorial encounters in songbirds. In 58% of cases, more than one male responded to the song stimuli. Most responses of non-focal males were to complex song stimuli. Evidence suggests that longer, more complex songs function mainly for female attraction (Catchpole & Slater 2008), but also male-male territoriality (McGregor & Horn 1992). Therefore it is expected that mate attraction and rival repulsion are intrinsically linked, because a male that sings more complex songs is more likely to obtain a partner, and is therefore a tangible threat to any territorial male (Seddon *et al.* 2013).

An individual's level of aggression and fighting ability can reflect its physical performance capacities and its physiological state (Lailvaux & Irschick 2006). Aggressive encounters in the natural world can be highly ritualised or result in actual fighting (Alonso-Alvarez *et al.* 2003). It has been shown that females will also assess a male's potential physical fighting ability through a well-choreographed display when selecting a partner (Alonso-Alvarez *et al.* 2003), because fighting ability could reflect overall quality of the male, although the extent to which this occurs in Tui remains unknown. Indeed, many male songbirds use song and song matching as their main means of advertisement of quality to rival males and potential mates (Kroodsma 1979).

In this study, complex song evoked strong male responses with a limited amount of female response. It must be acknowledged that using songs from a single male may limit the conclusions that can be drawn from this study. For example, unknown and undetected characteristics of the male used to obtain the stimuli may have elicited certain response types. Future studies in this area should therefore consider these factors in the experimental design phase. Further studies examining how complex song varies with reproductive success of males and females in addition to male condition are also recommended to establish whether song complexity is an indicator of Tui male reproductive quality. Furthermore, the effect of song on females and the role of complex song in mate attraction in complex vocal species needs further consideration.

In summary, our results indicated that territorial male Tui responded more strongly to complex song than simple song stimuli during the breeding season. Complex song was perceived as a threat and appeared to act as a trigger for aggressive male-male encounters. Our findings set a platform for future studies on the relationship between Tui song complexity and both reproductive potential and male quality, measured in terms of neural constraints, genetic diversity, developmental stress levels and parasite loads.

We thank Auckland Council for issuing a permit to conduct research at Tawharanui Regional Park (CS40); our field assistants Tanja Binzegger, Maira Fessardi, Akshay Ogra, and Justine Sanderson; Ngati Manuhiri for their support of our research; and Maurice Puckett and Colin Wards for their assistance at Tawharanui Regional Park. We also thank Felicity Moore and Arianne Abelardo for technical support.

REFERENCES

Alonso-Alvarez, C., Doutrelant, C. & Sorci, G. 2004. Ultraviolet reflectance affects male-male interactions in the blue tit (*Parus caeruleus ultramarinus*). *Behav. Ecol.* **15**: 805–809

Anderson, M.J. 2001.A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **26**: 32–46

Anderson, M.J. & Walsh, D.C.I. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecol. Monogr.* **83**: 557–574

Beecher, M.D. & Brenowitz, E.A. 2005. Functional aspects of song learning in songbirds. *Trends Ecol. Evol.* **20**: 143–149.

Beecher, M.D. & Campbell, S.E. 2005. The role of unshared songs in singing interactions between neighbouring song sparrows. *Anim. Behav.* **70**: 1297–1304.

Bergquist, C.A.L. & Craig, J.L. 1988. Competitive asymmetries, status, and breeding success of Tui (Meliphagidae) at an established feeding station. *N. Z. J. Zool.* **15**: 369–380.

Bergquist, C.A.L. 1989. *Tui sociodynamics: foraging behaviour, social organisation and use of song by Tui in an urban area*. PhD thesis. University of Auckland.

Boogert, N.J., Giraldeau, L-A. & Lefebvre, L. 2008. Song complexity correlates with learning ability in zebra finch males. *Anim. Behav.* **76**: 1735–1741.

Brunner, P. & Pasinelli, G. 2010. Variation in singing style use in the reed bunting *Emberiza schoeniclus*: influencing factors and possible functions. *J. Avian Biol.* **41**: 388–397.

Buchanan, K.L. & Catchpole, C.K. 2000. Song as an indicator of male parental effort in the sedge warbler. *Proc. Biol. Sci.* **267**: 321–326.

Campbell, D.L.M., Weiner, S.A., Starks, P.T. & Hauber, M.E. 2009. Context and control: behavioural ecology experiments in the laboratory. *Ann. Zool. Fennici*. **46**: 112–123.

Catchpole, C.K. 1980. Sexual Selection and the Evolution of Complex Songs among European Warblers of the Genus Acrocephalus. *Behaviour* **74**: 149–166.

Catchpole, C.K., Dittami, J. & Leisler, B. 1984. Differential responses to male song repertoires in female songbirds implanted with oestradiol. *Nature* **312**: 563–564.

Catchpole, C.K. & Leisler, B. 1996. Female aquatic warblers (*Acrocephalus paludicola*) are attracted by playback of longer and more complicated songs. *Behaviour* **133**: 1153–1164.

Catchpole, C.K. & Slater, P.J.B. 2008. *Bird Song: Biological Themes and Variations 2nd edition*. Cambridge: Cambridge University Press.

Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Austral Ecology*. 18: 117–143.

Cody, M.L., Stabler, E., Castellanos, H.M.S. & Taylor, C.E. 2016. Structure, syntax and "small-world" organization in the complex songs of California thrashers (*Toxostoma redivivum*). *Bioacoustics* 25: 1–14.

Cramer, E.R.A. 2013. Physically challenging song traits, male quality, and reproductive success in house wrens. *PLOS ONE* **8**: e59208.

DeVoogd, T.J., Krebs, J.R., Healy, S.D. & Purvis, A. 1993. Relations between song repertoire size and volume of brain nuclei related to song: comparative evolutionary analyses amongst oscine birds. *Proc. R. Soc. Lond. B.* **254**: 75–82.

DeVoogd, T.J. 2004. Neural constraints on the complexity of avian song. *Brain Behav. Evol.* **63**: 221–232.

Eliassen, S, Jørgensen, C. 2014. Extra-pair mating and evolution of cooperative neighbourhoods. *PLOS ONE* **9**: e99878.

Ewenson, E.L., Zann, R.A. & Flannery, G.R. 2001. Body condition and immune response in wild zebra finches: effects of capture, confinement and captive-rearing. *Naturwissenschaften* **88**: 391–394.

Falls, J.B. 1992. Playback: a historical perspective. In McGregor, P.K. (ed.) *Playback and studies of animal communication*: 11–33. New York: Plenum Press.

Feßl, B., & Hoi H. 2000. Song complexity and song structure in the moustached warbler *Acrocephalus melanopogon*. *J. Avian Biol.* **31**: 144–150.

Fischer, J., Noser, R. & Hammerschmidt, K. 2013. Bioacoustic Field Research: A primer to acoustic analyses and playback experiments with primates. *Am. J. Primatol.* **75**: 643–663.

Garamszegi, L.Z. & Møller, A.P. 2003. Extrapair paternity and the evolution of bird song. *Behav. Ecol.* 15: 508–519.

Garamszegi, L.Z. & Avilés, J.M. 2005. Brood parasitism by brown-headed cowbirds and the expression of sexual characters in their hosts. *Oecologia* **143**: 167–177.

Garamszegi, L.Z., Moreno, J. & Møller, A.P. 2006. Avian song complexity is associated with high field metabolic rate. *Evol. Ecol. Res.* 8: 75–90.

Greig-Smith, P.W. 1982. Seasonal patterns of song production by male stonechats, *Saxicola torquata*. *Ornis Scand.* **13**: 225–231.

Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? *Science* **218**: 384–387.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4(1).

Hasselquist, D., Bensch, S. & Schantz, T.V. 1996. Correlation between male song repertoire, extra pair paternity and offspring survival in the great reed warbler. *Nature* **381**: 229–232.

Hill, S.D. 2011. *The vocalisation of Tui (Prosthemadera novaeseelandiae*). MSc thesis. Massey University

Hill, S.D., Ji, W., Amiot, C., Parker, K.A. & Wells, S.J. 2013. A comparison of vocalisations between mainland Tui (*Prosthemadera novaeseelandiae novaeseelandiae*) and Chatham Island Tui (*P. n. chathamensis*). *N. Z. J. Ecol.* **37**: 214–223.

Hill, S.D. & Ji, W. 2013. Microgeographic variation in song phrases of Tui (*Prosthemadera novaeseelandiae*). *Notornis* 60: 262–264.

Hill, S.D., Ludbrook, M.R., Amiot, C. & Ji, W. 2015. Seasonal variation in the song structure of Tui (*Prosthemadera novaeseelandiae*). *N. Z. J. Ecol.* **39**: 110–115.

Hjernquist, M.B., Thuman Hjernquist, K.A., Forsmana, J.T. & Gustafsson, L. 2008. Sex allocation in response to local resource competition over breeding territories. *Behav. Ecol.* **20**: 335–339.

Howard, R.D. 1974. The influence of sexual selection and interspecific competition on mocking bird song (*Mimus polglottus*). *Evolution* 28: 428–438.

Jaška, P., Linhart, P. & Fuchs, R. 2015. Neighbour recognition in two sister songbird species with a simple and complex repertoire – a playback study. *J. Avian Biol.* **45**: 151–158.

Kipper, S., Kiefer, S., Bartsch, C. & Weiss, M. 2015. Female calling? Song responses to conspecific call playbacks in nightingales, *Luscinia megarhynchos*. *Anim. Behav.* **100**: 60–66.

Kokko, H. 1999. Cuckoldry and the stability of biparental care. *Ecol. Lett.* **2**: 247–255.

Kroodsma, D.E. 1979. Vocal dueling among male marsh wrens: evidence for ritualized expressions of dominance/subordinance. *Auk* **96**: 506–515.

Kroodsma, D.E. 1981. Geographical variation and functions of song types in warblers (Parulidae). *Auk* **98**: 743–751.

Kroodsma, D.E. 1990. Using appropriate experimental designs for intended hypotheses in 'song' playbacks, with examples for testing effects of song repertoire sizes. *Anim. Behav.* **40**: 1138–1150.

Kroodsma, D.E. & Byers, B.E. 1991. The function(s) of bird song. *Am. Zool.* **31**: 318–328.

Lailvaux, S.P. & Irschick, D.J. 2006. A functional perspective on sexual selection: insights and future prospects. *Anim. Behav.* **72**: 263–273.

Leedale, A.E., Collins, S.A. & de Kort, S.R. 2015. Blackcaps (*Sylvia atricapilla*) increase the whistle part of their song in response to simulated territorial intrusion. *Ethology* **121**: 403–409.

Leisler, B., Beier, J., Staudter, H. & Wink, M. 2000. Variation in extra-pair paternity in the polygynous great reed warbler (*Acrocephalus arundinaceus*). *J. Ornithol.* **141**: 77– 84.

McGregor, P.K. & Horn, A.G. 1992. Strophe length and response to playback in great tits. *Anim. Behav.* **43**: 667–676.

Marler, P. & Tamura, M. 1962. Song 'dialects' in three populations of white-crowned sparrows. *Condor* **64**: 368–377.

Marshall, R.C., Buchanan, K.L. & Catchpole, C.K. 2003. Sexual selection and individual genetic diversity in a songbird. *Proc. R. Soc. Lond. B.* **270**: S248–250.

Mennill, D.J., Ratcliffe, L.M. & Boag, P.T. 2002. Female eavesdropping on male song contests in songbirds. *Science* 296: 873.

Moiron, M., González-Lagos, C. Slabbekoorn, H. & Sol, D. 2015. Singing in the city: high song frequencies are no guarantee for urban success in birds. *Behav. Ecol.* **26**: 843–850.

Monbureau, M., Barker, J.M., Leboucher, G. & Balthazart J. 2015. Male song quality modulates c-Fos expression in the auditory forebrain of the female canary. *Physiol. Behav.* **147**: 7–15.

Mountjoy, D.J. & Lemon, R.E. 1991. Song as an attractant for male and female European starlings, and the influence of song complexity on their response. *Behav. Ecol. Sociobiol.* **28**: 97–100.

Mountjoy, D.J. & Lemon, R.E. 1996. Female choice for complex song in the European starling: a field experiment. *Behav. Ecol. Sociobiol.* **38**: 65–71.

Nowicki, S. & Searcy, W.A. 2004. Song function and the evolution of female preferences why birds sing, why brains matter. *Ann. N.Y. Acad. Sci.* **1016**: 704–723.

Olabarria, C., Incera, M., Garrido, J. & Rossi, F. 2010. The effect of wrack composition and diversity on macrofaunal assemblages in intertidal marine sediments. *J. Exp. Mar. Biol. Ecol.* **396**: 18–26.

Parker, K.A., Hauber, M.E. & Brunton, D.H. 2010. Contemporary cultural evolution of a conspecific recognition signal following serial translocations. *Evolution* **64**: 2431–2441.

Potvin, D.A., Crawford, P.W., MacDougall-Shackleton, S.A., & MacDougall-Shackleton E.A. 2015. Song repertoire size, not territory location, predicts reproductive success and territory tenure in a migratory songbird. *Can. J. Zool.* **93**: 627–633.

Pryke, S.R. 2013. Bird contests: from hatching to fertilisation. In Hardy, H.C.W. & Briffa, M. (eds.) *Animal contests*: 287–303. Cambridge: Cambridge University Press.

Reid, J.M., Arcese, P., Cassidy, A., Hiebert, S.M., Smith, J.N.M, Stoddard, P.K., Marr, A.B. & Keller, L.F. 2004. Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. *Anim. Behav,* **68**: 1055–1063.

Ritchison, G. 1988. Song repertoires and the singing behavior of male northern cardinals *Wilson Bull.* **100**: 583–603.

Sasahara, K., Cody, M.L., Cohen, D. & Taylor, C.E. 2012. Structural design principles of complex bird songs: a network-based approach. *PLOS ONE* **7(9)**: e44436

Schmidt, K.L., Drew Moore, S., MacDougall-Shackleton, E.A., MacDougall-Shackleton, S.A. 2013. Early-life stress affects song complexity, song learning and volume of the brain nucleus RA in adult male song sparrows. *Anim. Behav.* **86**: 25–35.

Searcy, W.A. 1992. Song repertoire and mate choice in birds. *Am. Zool.* **32**: 71–80.

Searcy, W.A. & Andersson, M. 1986. Sexual selection and the evolution of song. *Ann. Rev. Ecol Syst.* **17**: 507–533.

Searcy, W.A. & Yasukawa, K. 1990. Use of the song repertoire in intersexual and intrasexual contexts by male red-winged blackbirds. *Behav. Ecol. Sociobiol.* **27**: 123–128.

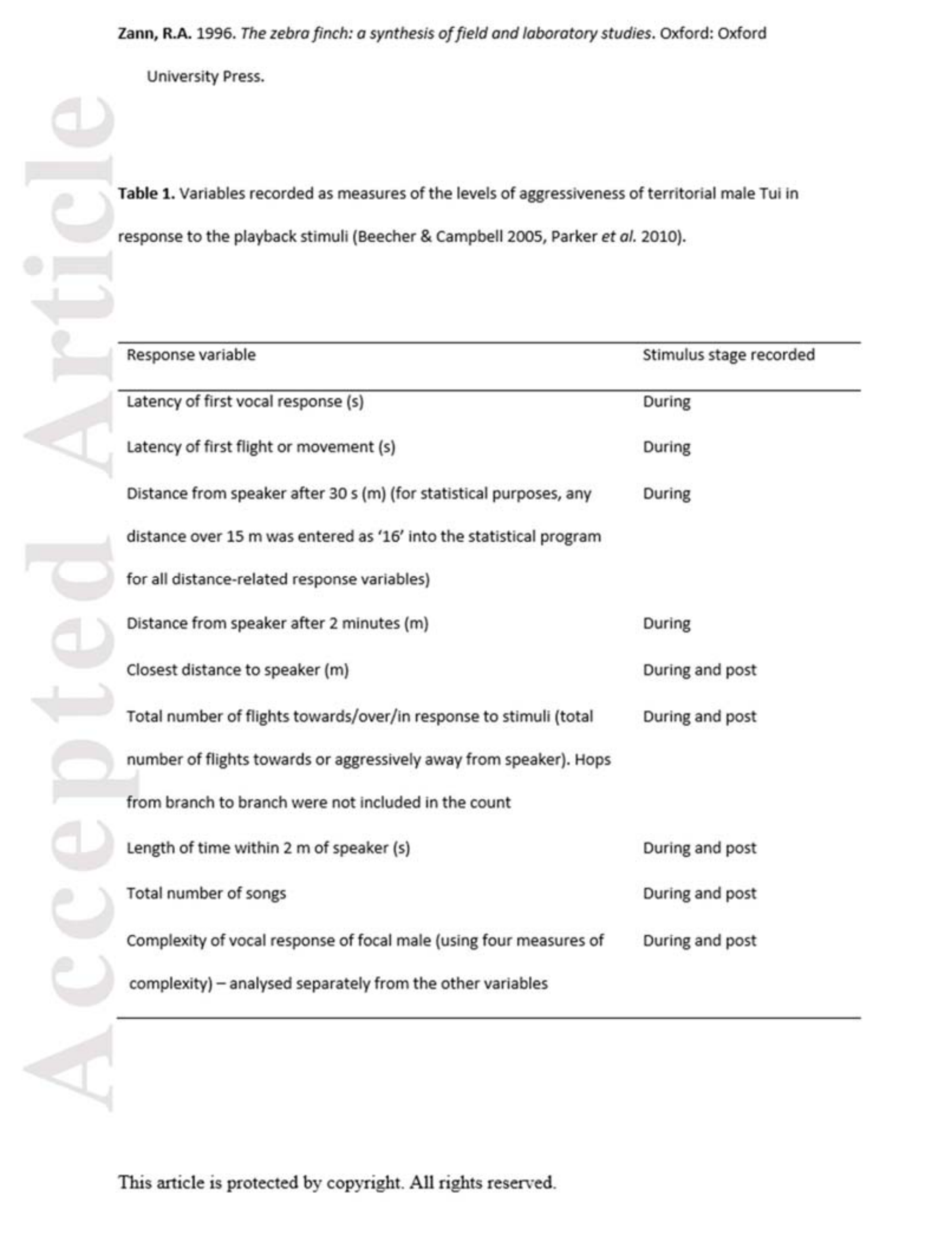
Searcy, W.A., Nowicki, S. & Hughes, M. 1997. The response of male and female song sparrows to geographic variation in song. *Condor* **99**: 651–657.

Seddon, N., Botero, C.A., Tobias, J.A., Dunn, P.O., MacGregor HEA Rubinstein D.R., Uy, J.A.C., Weir, J.T., Whittingham, L.A. & Safran, R.J. 2013. Sexual selection accelerates signal evolution during speciation. *Proc. R. Soc. Lond. B.* **280**: 20131065.

Spencer, K.A, Buchanan, K.L., Goldsmith, A.R. & Catchpole, C.K. 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Horm. Behav.* **44**: 132–139.

Wells, S.J., Ji, W., Dale, J., Jones, B. & Gleeson, D. 2015. Male size predicts extrapair paternity in a socially monogamous bird with extreme sexual size dimorphism. *Behav. Ecol.* **26**: 200–206.

West, M.J., King, A.P. & Eastzer, D.H. 1981. Validating the female bioassay of cowbird song: relating differences in song potency to mating success. *Anim. Behav.* **29**: 490–501.



Zann, R.A. 1996. *The zebra finch: a synthesis of field and laboratory studies*. Oxford: Oxford University Press.

Table 1. Variables recorded as measures of the levels of aggressiveness of territorial male Tui in response to the playback stimuli (Beecher & Campbell 2005, Parker *et al.* 2010).

Response variable	Stimulus stage recorded
Latency of first vocal response (s)	During
Latency of first flight or movement (s)	During
Distance from speaker after 30 s (m) (for statistical purposes, any distance over 15 m was entered as '16' into the statistical program for all distance-related response variables)	During
Distance from speaker after 2 minutes (m)	During
Closest distance to speaker (m)	During and post
Total number of flights towards/over/in response to stimuli (total number of flights towards or aggressively away from speaker). Hops from branch to branch were not included in the count	During and post
Length of time within 2 m of speaker (s)	During and post
Total number of songs	During and post
Complexity of vocal response of focal male (using four measures of complexity) – analysed separately from the other variables	During and post

Table 2. Eigenvalues, variance explained and factor loadings of the response variables following principal component analysis (PCA) for the response of focal males to complex, simple or control stimuli. *Variables with factor loading values greater than 0.7.

	PC1	PC2
Eigenvalue	5.73	0.99
% of variance	71.69	12.37
Latency of first response to stimuli (s)	0.91*	0.30
Latency of first flight or movement towards the speaker (s)	0.91*	0.29
Distance from speaker after 30 s (m)	0.88*	-0.06
Distance from speaker after 2 minutes (m)	0.84*	-0.32
Closest distance to speaker (m)	0.95*	0.12
Total number of flights towards/over/in response to stimuli	0.83*	-0.37
Length of time within 2 m of speaker (s)	-0.63	0.71*
Total number of songs during and post-stimuli	-0.78*	0.24

Table 3. The descriptive statistics including the means ± standard error values and *P* values from the Wilcoxon matched-pairs signed ranks test of the two variables that contributed the most variance in the PCA for all three stimuli types. Also shown are the mean PC1 scores for each treatment. Note that only the *P* values for complex vs simple song are shown.

Variable	Control song	Complex song	Simple song	*Z*	*P*
Closest distance to speaker (m)	15.1 ± 0.9	0.3 ± 0.2	6.3 ± 1.6	-2.807	0.005
Total number of songs during and post-stimuli	1 ± 0	15 ± 3	7 ± 2	-1.649	0.099
PC1 score	1.2 ± 0.1	-1 ± 0.1	-0.2 ± 0.2	-2.824	0.005

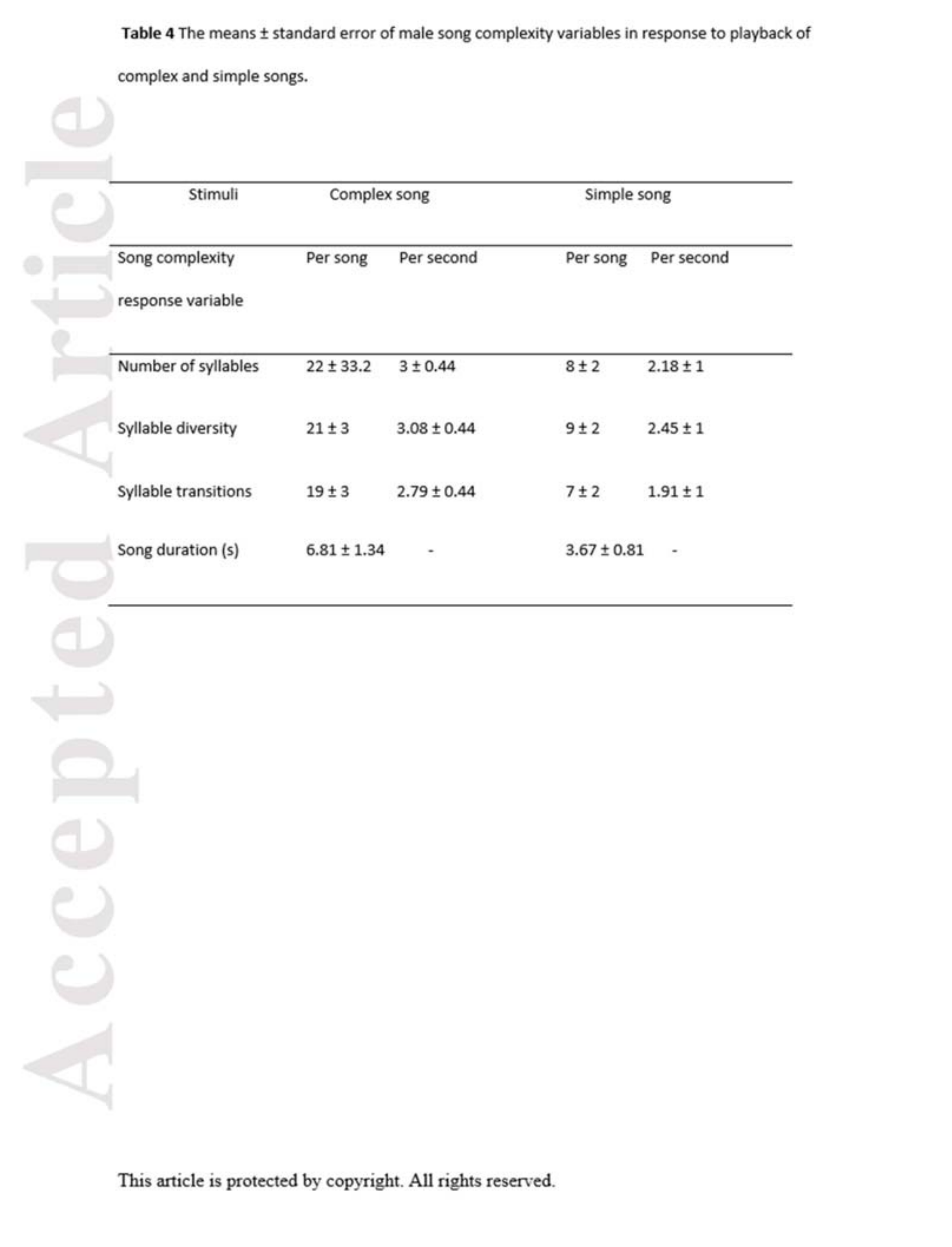


Table 4 The means ± standard error of male song complexity variables in response to playback of complex and simple songs.

Stimuli	Complex song		Simple song	
Song complexity response variable	Per song	Per second	Per song	Per second
Number of syllables	22 ± 33.2	3 ± 0.44	8 ± 2	2.18 ± 1
Syllable diversity	21 ± 3	3.08 ± 0.44	9 ± 2	2.45 ± 1
Syllable transitions	19 ± 3	2.79 ± 0.44	7 ± 2	1.91 ± 1
Song duration (s)	6.81 ± 1.34	-	3.67 ± 0.81	-

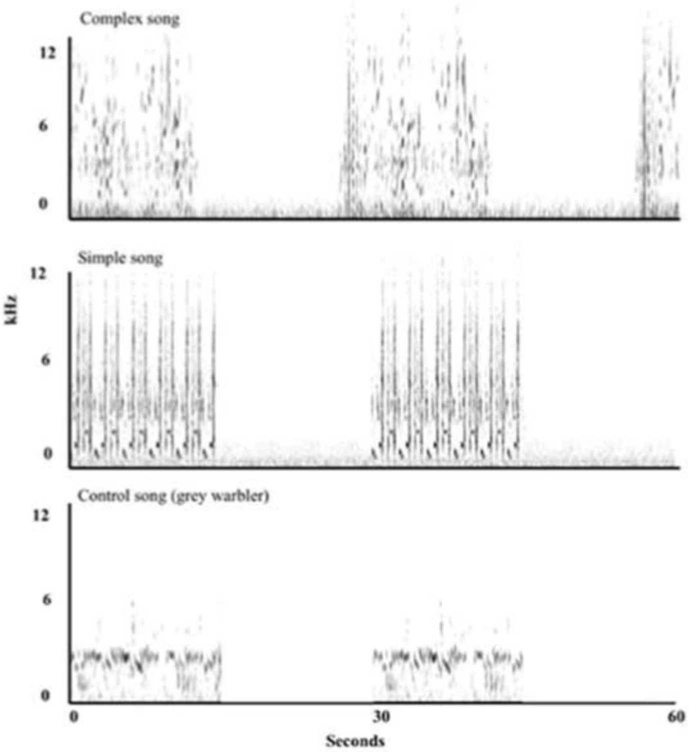


Figure 1. Sixty second exemplars of the song stimuli with different levels of complexity (the stimuli): complex song, simple song and control heterospecific song (Grey Warbler) used in these playback experiments. Each period of signal represents a single song bout and each song stimulus had a concordant signal-to-silence ratio.

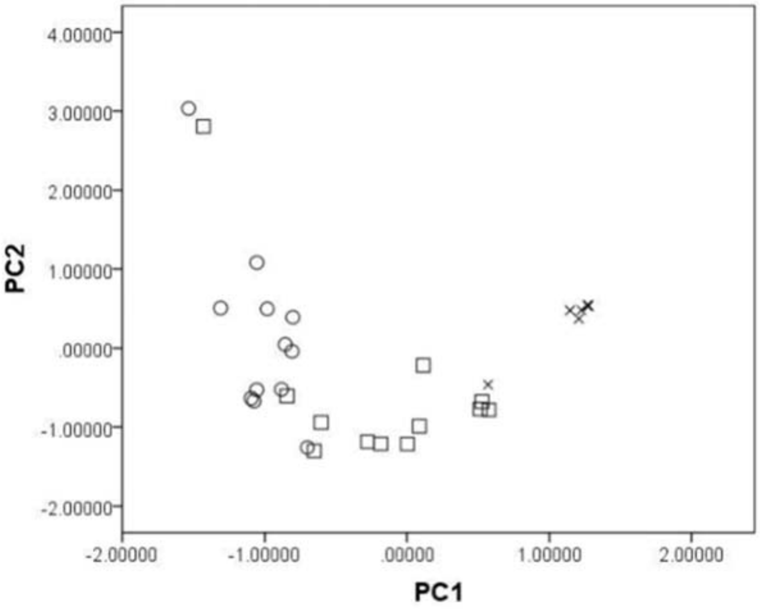


Figure 2. PCA scatterplot with Eigenvalue scales along the axes (circles: complex song, squares: simple song, crosses: control heterospecific song).

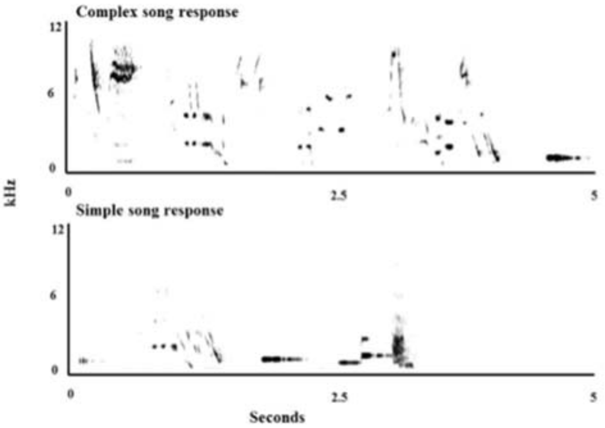


Figure 3. An exemplar of Tui song responses to complex song and simple song playback.

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Local habitat complexity correlates with song complexity in a vocally elaborate honeyeater

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Abstract Song complexity is an important behavioural trait in songbirds, subject to sexual selection. Elucidation of intraspecific variation in song complexity can provide insights into its evolution. In this study, we investigated song complexity variation in tūī (*Prosthemadera novaeseelandiae*), a vocally complex songbird endemic to New Zealand. At two separate nature reserves, we recorded male songs in two habitat types: forest remnants with high habitat complexity, and open habitats with lower habitat complexity. Analyses indicated strong evidence that song complexity was higher in forest habitats. Possible explanations for this divergence include: (i) competition between individuals results in higher quality, dominant males with more complex songs occupying forest habitats, and less competitive males occupying open habitat zones; (ii) forest habitats provide more abundant resources therefore higher tūī density, resulting in more complex songs; and (iii) a higher abundance of food in dense forest habitats may reduce nutritional stress during development resulting in full development of song nuclei. However, these hypotheses on the drivers of habitat effects on tūī song complexity remain to be tested.

Key words: habitat complexity, singing behaviour, song complexity, songbird, tūī.

INTRODUCTION

In songbirds, the principal functions of song are territory defence (de Kort *et al.* 2009) and mate attraction (Briefer *et al.* 2010). Understanding ecological factors that influence intraspecific variation in sexually selected traits, such as song complexity, can provide important evolutionary insights.

Complex songs are costly to produce (Searcy 1992). Evidence suggests song complexity is a proxy for male fitness and a product of sexual selection and intrasexual competition (Spencer *et al.* 2004; Spencer & MacDougall-Shackleton 2011). Varying degrees of song complexity was found to influence male mating success (Hasselquist *et al.* 1996). Males with more complex songs tend to establish breeding territories earlier than males that produce less complex songs (Alatalo *et al.* 1986).

Song complexity can be measured using several variables such as syllable diversity (Grunst *et al.* 2016), song length (Gil & Gahr 2002) and trill proportion (Leitão *et al.* 2006). Trills are honest signals of male quality (Hennin *et al.* 2009). Both trills and songs are longer in closed and dense habitats (Wiley 1991; Van Dongen & Mulder 2006). Acoustic adaptation to divergent habitats can influence intraspecific communication and generate phenotypic variation in song complexity characteristics (Handford & Nottebohm 1976; Tubaro *et al.* 1993). However, there is no strong evidence that other song complexity measures such as syllable diversity and entropy vary in different habitats.

Tūī (*Prosthemadera novaeseelandiae*) are endemic New Zealand songbirds within the Meliphagidae (honeyeaters). They exhibit sexual size dimorphism and have high extrapair paternity (Wells *et al.* 2015). Natal philopatry and limited dispersal also occur in tūī (Bergquist 1985; Stewart & Craig 1985). This species is a good model for investigating intraspecific variation in song complexity since they colonize various habitat types and produce complex vocalizations (Hill *et al.* 2013, 2015). Previous studies found that mainland tūī songs were more complex than on remote islands (Hill *et al.* 2013). There is also evidence for differences in tūī song phrases at the microgeographic level (Hill & Ji 2013). However, whether tūī song complexity varies at the microgeographic level is still open to speculation. Using tūī as a model, within two nature reserves, we compared song complexity within two local habitat types with divergent levels of habitat complexity: (i) native forest fragments with more complex plant communities (forest); and (ii) habitat zones consisting of pasture and sporadic trees (open). We predicted higher song complexity in forest habitats than in open habitats,

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giving rise to microgeographic song complexity variation.

METHODS

Study sites

Tūī song recordings were collected from both closed forest remnants and open habitats, at two rural study sites in northern New Zealand: Tawharanui Regional Park and Wenderholm Regional Park.

Tawharanui is a 588 ha predator-free nature reserve 90 km north of central Auckland, New Zealand. The forest site here, Ecology Bush (36°37′S, 174°84′E; approximately 70 ha), consists predominantly of mature coastal forest remnants. Tree species include important food resources for tūī (e.g. puriri *Vitex lucens*, rewarewa *Knightia excelsa* and tawa *Beilschmiedia tawa*) with patches of regenerating scrub such as kanuka (*Kunzea robusta*, Hill *et al.* 2013). The open habitat study site at Jones Bay (36°37′S, 174°82′E; approximately 59 ha), approximately 2 km from Ecology Bush, consists of large open pasture with small patches or isolated trees. Important food plant species for tūī in the open habitat include native trees (e.g. kanuka, karo *Pittosporum crassifolium* and pohutukawa *Metrosideros excelsa*), and exotic species such as flame trees (*Erythrina x sykesii*).

Wenderholm is a 134 ha predator-controlled nature reserve 46 km north of central Auckland, New Zealand. The park sits bounded by two tidal estuaries with an 80 ha hilly forested headland. The two sampled habitats at Wenderholm lay approximately 1 km apart. The forest area [36°54′S, 174°71′E] features important food resource species for tūī such as kanono (*Coprosma grandifolia*), karamu (*Coprosma robusta*) and totara (*Podocarpus totara*). The open habitat [36°53′S, 174°71′E]) predominantly contains tūī food resource species such as houpapa (*Pseudopanax lessonii*), karo and pohutukawa.

Data analysis

Habitat complexity

To quantify habitat complexity, we measured vegetation structure and plant diversity. At each site we conducted vegetation surveys along 4 × 140 m transects (on pre-determined bearings using a compass), using the point-centred quarter (PCQ) method (Cottam & Curtis 1956), with 20 m intervals between centres of PCQ survey points. At each sampling point, a 'cross' using 2 × 1 m aluminium tubes was placed on the ground to create four quarters. At each quarter, the tree or shrub with a diameter at breast height (DBH, 1 m 35 cm) of ≥7.85 cm (2.5 π cm, measured using diameter tape) closest to the centre PCQ point was selected to measure. Established forestry protocol states that trees ≤7.85 cm are considered saplings (e.g. Vargas-Rodriguez *et al.* 2005). The plant species, DBH and the distance from the quarter to the centre point of the tree were recorded.

Three variables were employed to measure habitat complexity: (i) distance between the plant measured and the centre of the PCQ, which gave a measure of plant density; (ii) plant species richness, the total number of species recorded during the PCQ survey at each habitat (Gotelli & Colwell 2010) and (iii) DBH, measured for each individual tree, which is an indicator of tree size and maturity. Numerical habitat complexity data were normalized using a Z-score transformation. A one-way multivariate permutational analysis of variance (PERMANOVA) (Anderson 2001) using Euclidean distance and 9999 permutations was conducted to examine differences in habitat density between the forest and open habitat zones at both nature reserves.

Sound recordings

A Marantz (Marantz, Kanagawa, Japan) PMD620 solid-state digital recorder was used to record songs along with a Sennheiser (Sennheiser, Old Lyme, CT, USA) ME67 shotgun long-range ultradirectional microphone. Ultradirectional microphones are advantageous over omnidirectional microphones for recording focal individuals as they permit the procurement of high-quality recordings with minimal peripheral noise. All songs were recorded at a sampling frequency of 44.1 kHz, and at a resolution of 24-bits. The frequency response range of the long-range microphone was 40 Hz to 20 kHz.

For each habitat, we recorded adult male songs over a full day during tūī breeding season (in October 2012) between 0550 h (1 h before official sunrise) and 2100 h (1 h after official sunset). Recordings were made in calm and dry climatic conditions. Recording days were divided into three separate 3 h recording sessions: dawn (starting recording 1 h before official sunrise until 2 h after official sunrise), solar noon (starting recording 1.5 h before official solar noon until 1.5 h after official solar noon) and dusk (starting recording 2 h before official sunset until 1 h after official sunset). This ensured a standardized 9 h of recording effort in each habitat. During recording sessions, territorial tūī were located along a pre-determined 150 m line in each habitat. This line bisected the habitat survey transect lines described above. When a tūī was located, we initiated recording. Recording was terminated when the tūī ceased singing or vacated its singing perch. During each recording the location, time, unique band combination (if applicable), behaviour and song type of tūī were documented. For unbanded birds, only one individual was recorded during any one recording bout. Individuals generally were recorded more than once throughout each day. During the breeding season when tūī rigorously defend nests, breeding partners and flowering plants, males have a propensity to be bound to their territories (Hill *et al.* 2015). Observations of colour-banded tūī suggest some individual males will occupy the same territories for at least three successive breeding seasons (S. Hill, personal observation, 2015; S. Wells, personal communication, 2015). Territories were identified to the best of our ability. Resident males actively expelled intruding males from their territories. Although the possibility of male tūī moving between

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sampling locations during the breeding season cannot be excluded, we expect the likelihood to be low.

The recordings were made at a distance of between 2 m and 10 m from each focal bird. A song was defined as a continuous singing sequence with a silent period of ≥3 s before and after another singing sequence (e.g. Woolley & Rubel 1997). Only song recordings with good signal-to-noise ratios were analysed (≥10 db). If significant background noise from vocalizations, climatic factors or other sources were present on a song spectrogram, it was not included in the analysis. Furthermore, low-frequency background noise (≤0.4 kHz) was excluded from every song analysed. This was done without clipping any focal bird's signal on the recordings (therefore, no song measurements were affected). At Tawharanui, nine different individual tūī were recorded in Ecology Bush and seven at Jones Bay (16 in total). At Wenderholm, six tūī were recorded in the Wenderholm forested area and five in the Wenderholm open habitat (11 in total) (Table 1).

Twelve of the 16 recorded tūī at Tawharanui were uniquely colour-banded. In total, 625 tūī songs were included in the analyses across the two nature reserves (Table 1). Both male and female tūī produce songs, however, females sing far less frequently. Tūī produce two main types of song. Firstly, broadcast songs, vocalized from an elevated singing post to no obvious conspecific audience (Hill *et al.* 2013). Secondly, interactive songs that are produced when another tūī is in proximity. Male tūī broadcast song is the most frequent song type produced and can generally be recorded for every singing male. Female and male interactive songs on the other hand are significantly less frequent and cannot necessarily be sampled during recording sessions. There is evidence that broadcast and interactive songs also structurally differ (S. Hill, unpublished data, 2016). Consequently, only male broadcast songs were included in the analysis. The song spectrograms were digitized and variables measured using the Raven Pro 1.4 Beta software (Cornell Lab of Ornithology, Ithaca, NY, USA). For all recordings, spectrograms were created by Discrete Fourier Transform (DFT) with a Hann window. The frame length was 256 points, and we used a 50% frame overlap with a hop size of 2.9 ms, frequency grid spacing of 172 Hz and the bandwidth was set at 3 dB. Signal-to-noise ratios were measured on Raven Pro using the 'band limited energy detector' function.

Comparison of song complexity between habitats

Five song complexity variables (Table 2; Fig. 1) were extracted from each broadcast song recorded using Raven Pro. We averaged song variables for each individual tūī for dawn, solar noon and dusk songs. Three individuals produced songs at two times of day (two individuals during dawn and dusk; and one individual during solar noon and dusk), and 24 individuals at just one time of day (12 individuals during dawn; nine during solar noon; and three during dusk), resulting in 30 different 'individual-by-time' combinations. We used a canonical analysis of principal coordinates (CAP) (Anderson & Willis 2003) to determine whether the five variables measuring song complexity were sufficient to discriminate between songs from forest and open habitats. The CAP was run in PRIMER v.6 (Clarke & Gorley 2006) using the 30 averaged values (described above). A 'leave-one-out' cross-validation assessed the discriminatory power of the model. We calculated the leave-one-out cross-validation results by removing a single individual-by-time averaged song from the data, and classifying its habitat using only its song complexity characteristics. We

Table 1. The number of recorded birds, the number of songs recorded (625 songs in total), mean songs recorded per individual and the sampling effort in each habitat

	Ecology Bush	Jones Bay	Wenderholm forest	Wenderholm open
Total number of birds recorded	9	7	6	5
Total number of songs analysed	166	251	104	104
Mean songs per individual (± SE)	18 ± 3	36 ± 9	17 ± 5	21 ± 4
Total recording effort (hr)	9	9	9	9

Table 2. Variables employed in this study to measure song complexity

Variable	Definition	References
Syllable diversity	Number of different syllables per song	Grunst *et al.* (2016)
Song duration (s)	The length of song	Gil and Gahr (2002)
Trill length (s) and trill percentage	The length, and proportion, of song that contains high-frequency trill syllables	Brumm and Slater (2006)
Entropy	A unitless measure of song disorder/unpredictability. It measures spectral complexity. White noise, for example has a high entropy score, a pure tone has a low entropy score $H_{sel} = \sum_{f=f_1}^{f_2} \left(\frac{E_{bin}}{E_{sel}} . \log_2 \left(\frac{E_{bin}}{E_{sel}} \right) \right)$	Charif *et al.* (2010)

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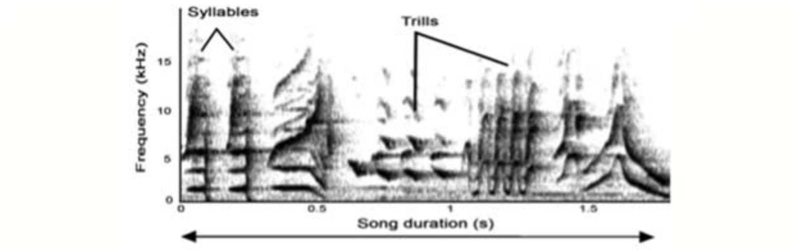


Fig. 1. A diagrammatic representation of the variables employed in this study to measure song complexity in tūī.

repeated this process for every song and the proportion of songs that were misclassified was calculated.

We used a one-way PERMANOVA using the Euclidean distance measure to test if there was evidence of a difference in song complexity between the forest and open habitats, using the same five song complexity measures. Values for each individual were averaged for the PERMANOVA as per the CAP analysis, with 9999 permutations. The song complexity values were averaged as per the CAP analysis. PERMANOVA tests in this study were conducted using the PERMANOVA+ add-on package for PRIMER.

RESULTS

Habitat complexity

The three variables of habitat complexity: distance of nearest tree or shrub from PCQ, plant species richness and DBH at each PCQ, were significantly dissimilar between forest habitats and open habitats at both Tawharanui and Wenderholm (PERMANOVA: $n_{forest} = 512$, $n_{open} = 512$; *Pseudo* $F_{1020,1024} = 128.9$; $P < 0.001$). Overall, habitat structure was more complex in the forest habitats including a higher density of plants with a DBH ≥ 7.85 cm, and greater plant species richness than the open habitats (Table 3).

Song complexity

For both Tawharanui and Wenderholm, the overall misclassification error was 6.7%. This means the CAP model chose the correct habitat in 93.3% of songs analysed (Fig. 2). This strongly implied that song complexity variation between the two habitats existed at both nature reserves. Song complexity was significantly higher in the forest habitats at both locations using all five song complexity variables (PERMANOVA: *Pseudo* $F_{21,27} = 8.417$; $P = 0.002$) (Table 4).

DISCUSSION

We have presented strong evidence of tūī song complexity variation between two different local habitat types. Consistent with the hypothesis, in forest habitats with a more complex plant community, tūī syllable diversity was greater, entropy (spectral complexity) was higher, song duration longer and trills both longer and of higher proportion. This is the first study to provide evidence for habitat complexity correlating with syllable diversity and entropy in tūī.

Table 3. The habitat complexity descriptive statistics of two habitats at Tawharanui and Wenderholm. The number of trees and shrubs in each habitat relative to each PCQ is also presented

Measurement	Ecology Bush	Jones Bay	Wenderholm forest	Wenderholm open
Mean distance (± SE) from PCQ to nearest tree/shrub (cm)	439.71 ± 41.5	1263.12 ± 50.12	409.3 ± 19.16	1443.02 ± 41.5
Total plant species richness	24	8	24	9
Mean (± SE) DBH of tree (cm)	38.32 ± 5.84	9.64 ± 1.57	23.24 ± 2.42	50.35 ± 5.49
Number of trees with a DBH ≥ 7.35 cm within 20 m from each PCQ	113	64	128	69
Total number of trees and shrubs within 20 m from each PCQ (total: 256)	236	142	256	137

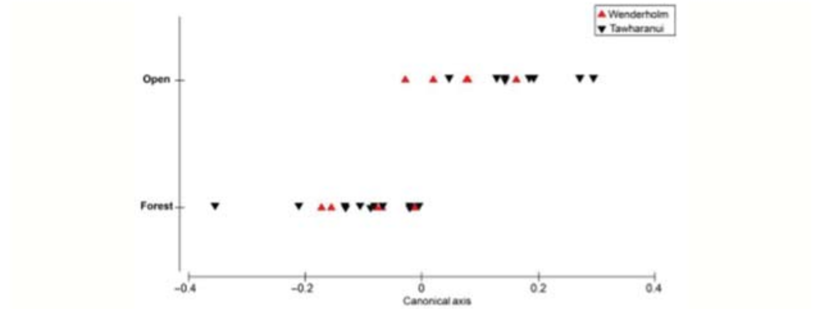


Fig. 2. A CAP analysis plot showing the forest and open habitats at both Tawharanui, and Wenderholm *vs.* the first canonical axis. The CAP plot chooses an axis that best discriminates the song complexity data and plots the data on that axis. The CAP analysis correctly classified 93.3% of the songs, strongly indicating that a high degree of variation in song complexity between the forest and open habitats existed in both nature reserves.

Table 4. The descriptive statistics of the five variables measuring song complexity in two tūī subpopulations at Tawharanui Regional Park and Wenderholm Regional Park

	Mean ± SE		Mean ± SE	
Variable	Ecology Bush	Jones Bay	Wenderholm forest	Wenderholm open
Syllable diversity	18.85 ± 0.91	16.1 ± 0.52	19.67 ± 1.33	17.1 ± 0.93
Song duration (s)	11.36 ± 0.78	6.23 ± 0.27	8.28 ± 0.78	6.68 ± 0.52
Trill length (s)	3.20 ± 0.21	1.45 ± 0.07	2.38 ± 1.79	1.76 ± 1.11
Trill percentage	31.19 ± 1.79	24.39 ± 1.1	32.33 ± 1.18	30.43 ± 1.13
Entropy (average)	3.43 ± 0.35	3.13 ± 0.29	3.89 ± 0.47	3.30 ± 0.24

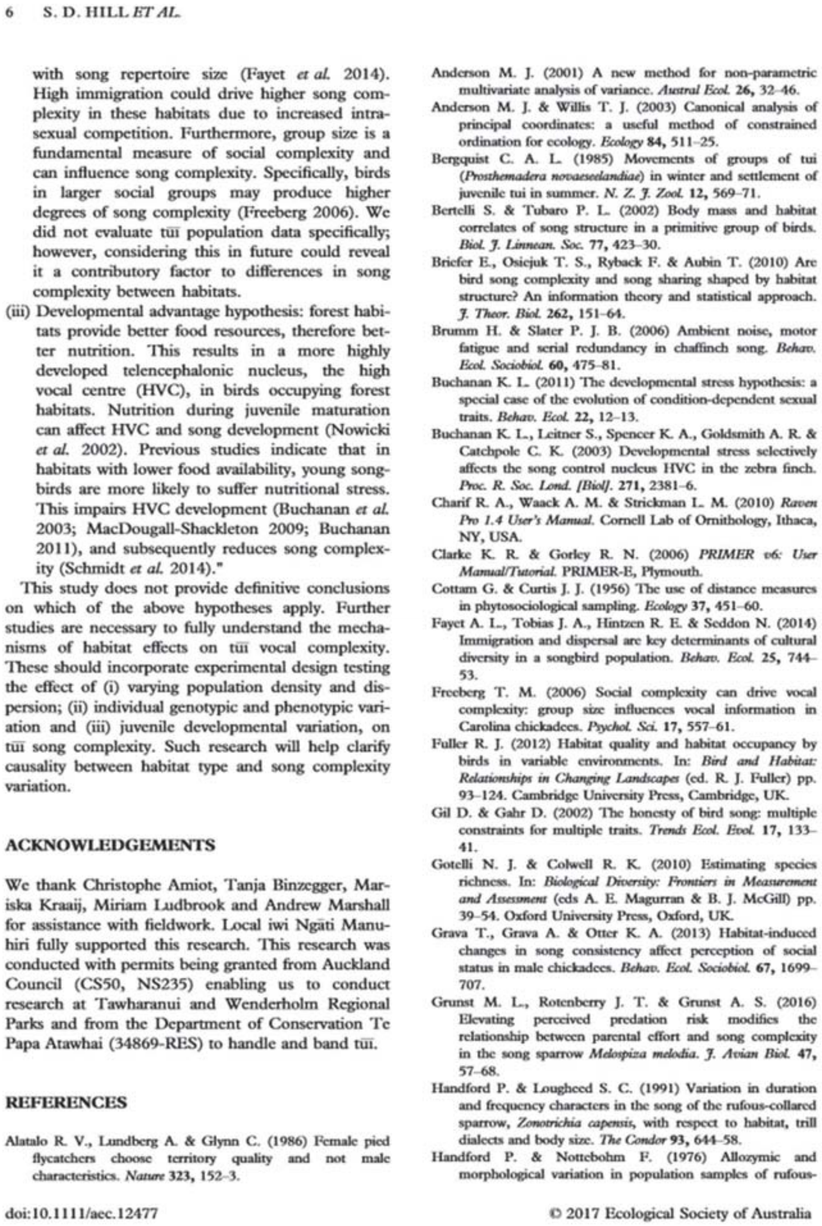
Longer song duration in forest habitats is consistent with previous research (Handford & Lougheed 1991; Van Dongen & Mulder 2006). Birds occupying territories with denser understoreys sing longer songs (Van Dongen & Mulder 2006). This suggests habitat variation contributes to intraspecific variation in song. Song length increases in more complex habitats are associated with increases in the number of syllables produced (Van Dongen & Mulder 2006).

Trill length increases in closed, dense forest habitats observed in tūī is also consistent with previous research (Wiley 1991) and likely relate to acoustic adaptation. Birds produce longer trills to mitigate reverberations in closed habitats that have numerous scattering surfaces such as leaves and branches (Tubaro & Segura 1994). This 'transmission fidelity' helps explain individual intraspecific differences in song variables observed in divergent habitat structures (Bertelli & Tubaro 2002; Nemeth *et al.* 2006).

There are various potential explanations for the observed correlation between song complexity and habitat complexity in tūī. We propose the following hypotheses:

(i) Intraspecific competition hypothesis: competition between male tūī for breeding territory results in more dominant individuals occupying forest habitats with more abundant food resources and more optimal nesting sites. Evidence indicates male songbirds with higher phenotypic quality will have higher song complexity (e.g. Schmidt *et al.* 2013). Furthermore, song complexity generally increases with both a male's resource holding potential (Grava *et al.* 2013), social status (Spencer *et al.* 2004) and competitive ability (Searcy & Nowicki 2005). When individuals vary in competitive ability, a 'despotic distribution' may be evident, where dominant individuals occupy superior habitats and less competitive individuals settle in marginal habitats (Fuller 2012).

(ii) Neighbour effect hypothesis: better quality habitats with more abundant food resources and nesting sites support higher densities of tūī. Therefore, individuals will have a greater number of neighbours. Current understanding indicates that high-quality habitats, attracting higher degrees of immigration, are positively associated



with song repertoire size (Fayet *et al.* 2014). High immigration could drive higher song complexity in these habitats due to increased intrasexual competition. Furthermore, group size is a fundamental measure of social complexity and can influence song complexity. Specifically, birds in larger social groups may produce higher degrees of song complexity (Freeberg 2006). We did not evaluate tūī population data specifically; however, considering this in future could reveal it a contributory factor to differences in song complexity between habitats.

(iii) Developmental advantage hypothesis: forest habitats provide better food resources, therefore better nutrition. This results in a more highly developed telencephalonic nucleus, the high vocal centre (HVC), in birds occupying forest habitats. Nutrition during juvenile maturation can affect HVC and song development (Nowicki *et al.* 2002). Previous studies indicate that in habitats with lower food availability, young songbirds are more likely to suffer nutritional stress. This impairs HVC development (Buchanan *et al.* 2003; MacDougall-Shackleton 2009; Buchanan 2011), and subsequently reduces song complexity (Schmidt *et al.* 2014)."

This study does not provide definitive conclusions on which of the above hypotheses apply. Further studies are necessary to fully understand the mechanisms of habitat effects on tūī vocal complexity. These should incorporate experimental design testing the effect of (i) varying population density and dispersion; (ii) individual genotypic and phenotypic variation and (iii) juvenile developmental variation, on tūī song complexity. Such research will help clarify causality between habitat type and song complexity variation.

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REFERENCES

Alatalo R. V., Lundberg A. & Glynn C. (1986) Female pied flycatchers choose territory quality and not male characteristics. *Nature* **323,** 152–3.

Anderson M. J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **26,** 32–46.

Anderson M. J. & Willis T. J. (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* **84,** 511–25.

Bergquist C. A. L. (1985) Movements of groups of tui (*Prosthemadera novaeseelandiae*) in winter and settlement of juvenile tui in summer. *N. Z. J. Zool.* **12,** 569–71.

Bertelli S. & Tubaro P. L. (2002) Body mass and habitat correlates of song structure in a primitive group of birds. *Biol. J. Linnean. Soc.* **77,** 423–30.

Briefer E., Osiejuk T. S., Ryback F. & Aubin T. (2010) Are bird song complexity and song sharing shaped by habitat structure? An information theory and statistical approach. *J. Theor. Biol.* **262,** 151–64.

Brumm H. & Slater P. J. B. (2006) Ambient noise, motor fatigue and serial redundancy in chaffinch song. *Behav. Ecol. Sociobiol.* **60,** 475–81.

Buchanan K. L. (2011) The developmental stress hypothesis: a special case of the evolution of condition-dependent sexual traits. *Behav. Ecol.* **22,** 12–13.

Buchanan K. L., Leitner S., Spencer K. A., Goldsmith A. R. & Catchpole C. K. (2003) Developmental stress selectively affects the song control nucleus HVC in the zebra finch. *Proc. R. Soc. Lond. [Biol].* **271,** 2381–6.

Charif R. A., Waack A. M. & Strickman L. M. (2010) *Raven Pro 1.4 User's Manual.* Cornell Lab of Ornithology, Ithaca, NY, USA.

Clarke K. R. & Gorley R. N. (2006) *PRIMER v6: User Manual/Tutorial.* PRIMER-E, Plymouth.

Cottam G. & Curtis J. J. (1956) The use of distance measures in phytosociological sampling. *Ecology* **37,** 451–60.

Fayet A. L., Tobias J. A., Hintzen R. E. & Seddon N. (2014) Immigration and dispersal are key determinants of cultural diversity in a songbird population. *Behav. Ecol.* **25,** 744–53.

Freeberg T. M. (2006) Social complexity can drive vocal complexity: group size influences vocal information in Carolina chickadees. *Psychol. Sci.* **17,** 557–61.

Fuller R. J. (2012) Habitat quality and habitat occupancy by birds in variable environments. In: *Bird and Habitat: Relationships in Changing Landscapes* (ed. R. J. Fuller) pp. 93–124. Cambridge University Press, Cambridge, UK.

Gil D. & Gahr D. (2002) The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol. Evol.* **17,** 133–41.

Gotelli N. J. & Colwell R. K. (2010) Estimating species richness. In: *Biological Diversity: Frontiers in Measurement and Assessment* (eds A. E. Magurran & B. J. McGill) pp. 39–54. Oxford University Press, Oxford, UK.

Grava T., Grava A. & Otter K. A. (2013) Habitat-induced changes in song consistency affect perception of social status in male chickadees. *Behav. Ecol. Sociobiol.* **67,** 1699–707.

Grunst M. L., Rotenberry J. T. & Grunst A. S. (2016) Elevating perceived predation risk modifies the relationship between parental effort and song complexity in the song sparrow *Melospiza melodia*. *J. Avian Biol.* **47,** 57–68.

Handford P. & Lougheed S. C. (1991) Variation in duration and frequency characters in the song of the rufous-collared sparrow, *Zonotrichia capensis*, with respect to habitat, trill dialects and body size. *The Condor* **93,** 644–58.

Handford P. & Nottebohm F. (1976) Allozymic and morphological variation in population samples of rufous-

doi:10.1111/aec.12477

collared sparrow, *Zonotrichia capensis*, in relation to vocal dialects. *Evolution* **30,** 802–17.

Hasselquist D., Bensch S. & von Schantz T. (1996) Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* **381,** 229–32.

Hennin H. L. N., Barker K. S., Bradley D. W. & Mennill D. J. (2009) Bachelor and paired male rufous-and-white wrens use different singing strategies. *Behav. Ecol. Sociobiol.* **64,** 151–9.

Hill S. D. & Ji W. (2013) Microgeographic variation in song phrases of tui (*Prosthemadera novaeseealandiae*). *Notornis* **60,** 262–4.

Hill S. D., Ji W., Parker K. A., Amiot C. & Wells S. J. (2013) A comparison of vocalisations between mainland tui (*Prosthemadera novaeseelandiae novaeseelandiae*) and Chatham Island tui (*P. n. chathamensis*). *N. Z. J. Ecol.* **37,** 214–23.

Hill S. D., Amiot C., Ludbrook M. R. & Ji W. (2015) Seasonal variation in the song structure of tui (*Prosthemadera novaeseelandiae*). *N. Z. J. Ecol.* **39,** 110–15.

de Kort S. R., Eldermire E. R. B., Cramer E. R. A. & Vehrencamp S. L. (2009) The deterrent effect of bird song in territory defense. *Behav. Ecol.* **20,** 200–6.

Leitão A., Ten Cate C. & Riebel K. (2006) Within-song complexity in a songbird is meaningful to both male and female receivers. *Anim. Behav.* **71,** 1289–96.

MacDougall-Shackleton S. A. (2009) The importance of development: what songbirds can teach us. *Can J. Psychol.* **63,** 74–9.

Nemeth E., Dabelsteen T., Pedersen S. B. & Winkler H. (2006) Rainforests as concert halls for birds: are reverberations improving sound transmission of long song elements. *J. Acoust. Soc. Am.* **119,** 620–6.

Nowicki S., Searcy W. A. & Peters S. (2002) Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". *J. Comp. Physiol.* **188,** 1003–14.

Schmidt K. L., Moore S. D., MacDougall-Shackleton E. A. & MacDougall-Shackleton S. A. (2013) Early-life stress affects song complexity, song learning and volume of the brain nucleus RA in adult male song sparrows. *Anim. Behav.* **86,** 25–35.

Schmidt K. L., MacDougall-Shackleton E. A., Kubli S. P. & MacDougall-Shackleton S. A. (2014) Developmental stress, condition, and birdsong: a case study in song sparrows. *Integr. Comp. Biol.* **54,** 568–77.

Searcy W. A. (1992) Song repertoire and mate choice in birds. *Integr. Comp. Biol.* **32,** 71–80.

Searcy W. A. & Nowicki S. (2005) *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton University Press, Princeton, NJ, USA.

Spencer K. A. & MacDougall-Shackleton S. A. (2011) Indicators of development as sexually selected traits: the developmental stress hypothesis in context. *Behav. Ecol.* **22,** 1–9.

Spencer K. A., Buchanan K. L., Goldsmith A. R. & Catchpole C. K. (2004) Developmental stress, social rank and song complexity in the European starling (*Sturnus vulgaris*). *Proc. R. Soc. Lond. [Biol]*. **271,** S121–3.

Stewart A. M. & Craig J. L. (1985) Movements, status, access to nectar, and spatial organisation of the tui. *N. Z. J. Zool.* **12,** 649–66.

Tubaro P. L. & Segura E. T. (1994) Dialect differences in the Song of *Zonotrichia capensis* in the Southern Pampas: a test of the acoustic adaptation hypothesis. *The Condor* **96,** 1084–8.

Tubaro P. L., Segura E. T. & Handford P. (1993) Geographic variation in the song of the rufous-collared sparrow in Eastern Argentina. *The Condor* **95,** 588–95.

Van Dongen W. F. D. & Mulder R. A. (2006) Habitat density, song structure and dialects in the Madagascar paradise flycatcher (*Terpsiphone mutata*). *J. Avian Biol.* **37,** 349–56.

Vargas-Rodriguez Y. L., Vazquez-Garcia J. A. & Williamson G. B. (2005) Environmental correlates of tree and seedling-sapling distributions in a Mexican tropical dry forest. 2005. *Plant Ecol.* **180,** 117–34.

Wells S. J., Ji W., Dale J., Jones B. & Gleeson D. (2015) Male size predicts extrapair paternity in a socially monogamous bird with extreme sexual size dimorphism. *Behav. Ecol.* **26,** 200–6.

Wiley R. H. (1991) Associations of song properties with habitats for territorial oscine birds of eastern North America. *Am. Nat.* **138,** 973–93.

Woolley S. M. N. & Rubel E. W. (1997) Bengalese finches *Lonchura striata domestica* depend upon auditory feedback for the maintenance of adult song. *J. Neurosci.* **17,** 6380–90.

Appendix 2: Extra-pair paternity frequencies

A summary of the 78 songbird species analysed in Chapter 2, with EPP frequency (% of all offspring), and the respective literature references. Only the literature references not cited within the thesis text are included in the reference list in this Appendix.

Species	Extra-pair paternity frequency	Reference
Malurus cyaneus	67	Dunn & Cockburn 1998
*Malurus splendens**	65	Tarvin et al. 2005
*Phylidonyris pyrrhopterus**	58	Petrie et al. 1998
Vireo olivaceus	57.89	Morton et al. 1998
*Prosthemadera novaeseelandiae**	57	Wells et al. 2015
Emberiza schoeniclus	55	Dixon et al. 1994
*Rhipidura albiscapa**	55	Hoffman et al. 2010
Turdus migratorius	53	Montgomerie in Briskie et al. 1997
Tachycineta bicolor	>50	Kempenaers et al. 2001
*Notiomystis cyncta**	46	Castro et al. 2004
Periparus ater	46	Schmoll et al. 2009
Phylloscopus fuscatus (M, P)	45	Petrie & Kempenaers 1998
*Caligavis chrysops**	44	Ewen et al. 2008
*Cracticus tibicen**	44	Durrant & Hughes 2005
Icterus galbula	37	Richardson & Burke 1999
Passerina cyanea	36.37	Westneat 1987
Acrocephalus paludicola	36	Schulze-Hagen et al. 1993
Emberiza citrinella	36	Sundberg & Dixon 1996
Zonotrichia leucophrys	36	Sherman & Morton 1988
Passerculus sandwichensis (P,M)	33.7	Freeman-Gallant 1997

*Setophaga petechia**	33.1	Yezerinac & Weatherhead 1997
Phylloscopus trochilus	33	Gyllensten et al. 1990
Wilsonia pusilla	32	Petrie & Kempenaers 1998
Empidonax virescens	30	Woolfenden et al. 2005
Hirundo rustica	28.8	Møller & Tegelström 1997; Saino et al. 1997
Junco hyemalis	27	Gerlach et al. 2012
Setophaga citrina	26.7	Stutchbury et al. 1994
*Melozone crissalis**	26	Benedict 2008
Nectarinia osea	26	Zilberman et al. 1999
Agelaius phoeniceus (M, P)	25.6	Westneat 1993
Melospiza melodia	24	Hill et al. 2011
Progne subis	23.5	Wagner et al. 1996a,b
*Setophaga caerulescens**	23.5	Webster et al. 2001
*Geothlypis trichas**	20	Garvin et al. 2006
Luscinia svecica	20	Krokene et al. 1996
*Sialia Mexicana**	20	Dickinson 2001
Delichon urbica	19	Whittingham & Lifjeld 1995
Calcarius ornatus	17.64	Hill & Gould 1997
*Euplectes orix**	17.6	Friedl & Klump 2002
Fringilla coelebs	17	Sheldon & Burke 1994
Turdus merula	17	Garamszegi et al. 2004
Spizella pusilla	15.1	Carey et al. 1994
Ficedula albicollis	15	Sheldon & Ellegren 1999
Dolichonyx oryzivorus	14.6	Gissing et al. 1998
Panurus biarmicus	14.4	Hoi & Hoi-Leitner 1997
Carduelis tristis	14.3	Bollinger & Gavin 1991
Zonotrichia albicollis	12.8	Tuttle 1993, 2003
Parus cristatus	12.4	Lens et al. 1997
Parus caeruleus	12.03	Leech et al. 2001
Sayornis phoebe	11.84	Conrad et al. 1998
Cardinalis cardinalis (occ. P)	11	Ritchison et al. 1994
Ficedula hypoleuca (M, P)	11	Canal et al. 2012

Oenanthe oenanthe	11	Currie et al. 1999
Passer domesticus	11	Stewart et al. 2006
Parus major	9.63	Otter et al. 2001
Passer montanus	9.06	Cordero et al. 2002
Parus atricapillus	8.91	Otter et al. 1998
Sturnus vulgaris (M, P)	8.7	Smith & von Schantz 1993
Troglodytes aedon	8.35	Soukup & Thompson 1997
Acrocephalus schoenobaenus	8	Marshall et al. 2007
Haemorhous mexicanus	8	Hill et al. 1994
Mimus polyglottos	8	Derrickson & Breitwisch 1992
Acrocephalus vaughni taiti ***	7	Brooke & Hartley 1995
Acanthiza pusilla	6.2	Green et al. 2002
Lanius collurio	5.26	Fornasari et al. 1994
Anthus spinoletta	5.23	Reyer et al. 1997
Carduelis flammea	4.5	Angst 1998
Emberiza calandra	4.5	Hartley et al. 1993, 1996
*Locustella luscinioides**	4.1	Neto et al. 2010
Erithacus rubecula	4	Tobias 1996
Acrocephalus arundinaceus (M, P)	3	Hasselquist et al. 1995
Acrocephalus palustris	3	Leisler & Wink 2000
Vireo solitarius	2.7	Morton et al. 1998
Taeniopygia guttata	2.4	Birkhead et al. 1990
Campylorhynchus nuchalis	1.4	Rabenold et al. 1990
Parus montanus	0.96	Orell et al. 1997
Calcarius pictus	0.9	Briskie et al. 1998
Prunella modularis	0.8	Burke et al. 1989

*Species not analysed in either Garamszegi & Møller (2004) nor Soma & Garamszegi (2011).

**Only one male recording available.

References (extra-pair paternity, mating system, parental care data)

Adkins-Regan E. 2002. Development of sexual partner preference in the zebra finch: a socially monogamous, pair-bonding animal. Archives of Sexual Behavior 31: 27–33.

Adkins-Regan E. 2013. Hormones and animal social behavior. Princeton University Press.

Ammon EM, Gilbert WM. 1999. Wilson's warbler (*Cardellina pusilla*). In: Poole A, Gill, F (eds). The Birds of North America, No. 478. The Academy of Natural Sciences, Philadelphia, PA, USA.

Angst C. 1998. Extra-pair paternity in the redpoll. In: Spina F, Grattarola A (eds). First meeting of the European Ornithologists' Union, Bologna, August 28–30, 1997. Istitutio Nazionale per la Fauna Selvatica “Alessandro Ghigi”, Bologna, Italy.

Banbura J, Perret P, Blondel J, Lambrechts MM. 2001. Sex differences in parental care in a Corsican blue tit *Parus caeruleus* population. Ardea-Wageningen 89: 517–526.

Bartlett TL, Mock DW, Schwagmeyer PL. 2005. Division of labor: incubation and biparental care in house sparrows (*Passer domesticus*). Auk 122: 835–842.

Best LB. 1977. Territory quality and mating success in the field sparrow (*Spizella pusilla*). Condor 79: 192–204.

Benedict L. 2008. Occurrence and life history correlates of vocal duetting in North American passerines. Journal of Avian Biology: 39: 57–65.

Birkhead TR, Burke T, Zann R, Hunter FM, Krupa AP. 1990. Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata*, revealed by DNA fingerprinting. Behavioral Ecology and Sociobiology 27: 315–324.

Bollinger EK, Gavin TA. 1991. Patterns of extra-pair fertilizations in bobolinks. Behavioral Ecology and Sociobiology 29: 1–7.

Bollmann K, Reyer H-U. 1999. Why does monogamy prevail in the alpine water pipit? In: Adams NJ, Slotow RH (eds). Proc. 22 International Ornithological Congress, Durban. BirdLife South Africa, Johannesburg. pp. 2666–2688.

Carey M, Burhans DE, Nelson DA. 2008. Tree sparrow (*Spizella pusilla*). In: Poole A, Gill F (eds). The birds of North America, no. 103. The Academy of Natural Sciences, Philadelphia. pp. 1–20.

Breitwisch R, Merritt PG, Whitesides GH. 1986. Parental investment by the Northern mockingbird: male and female roles in feeding nestlings. Auk 103: 152–159.

Breitwisch R, Schilling AJ, Banks JB. 1999. Parental behavior of a bigamous male northern cardinal. The Wilson Bulletin 111: 283–286.

Brenna E. 2007. Parental care in relation to paternity in the reed bunting (*Emberiza schoeniclus*) [PhD] University of Oslo, Oslo, Norway.

Briskie JV, Montgomerie R, Birkhead TR. 1997. The evolution of sperm size in birds. Evolution 51: 937–945.

Briskie JV, Montgomerie R Poldmaa T, Boag PT. 1998. Paternity and paternal care in the polygynandrous Smith's longspur. Behavioral Ecology and Sociobiology 43: 181–190.

Brooke MDL, Hartley IR. 1995. Nesting Henderson reed-warblers (Acrocephalus vaughani taiti) studied by DNA fingerprinting: unrelated coalitions in a stable habitat? Auk: 112: 77–86.

Buchanan KL, Catchpole CK. 2000. Song as an indicator of male parental effort in the sedge warbler. Proceedings of the Royal Society of London B: Biological Sciences 267: 321–326.

Burke T, Davies NB, Bruford MW, Hatchwell BJ. 1989. Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. Nature 338: 249–251.

Carnaby T. 2009. Beat about the bush: birds. Jacana Media (Pty) Ltd., Johannesburg, South Africa.

Castro I, Mason KM, Armstrong DP, Lambert DM. 2004. Effect of extra-pair paternity on effective population size in a reintroduced population of the endangered hihi, and potential for behavioural management. Conservation Genetics 5: 381–393.

Childs HE. Jr. 1968. The brown towhee. In: Austin Jr PL (ed). Life histories of North American cardinals, grosbeaks, buntings, towhees, finches, sparrows, and allies. U.S. National Museum Bulletin 237: 603–619.

Conrad KF, Robertson RJ, Boag PT. 1998. Frequency of extra-pair young increases in second broods of eastern phoebes. The Auk 115: 497–502.

Cordero PJ, Heeb P, Wetton JH, Parkin DT. 2002. Extra-pair fertilizations in tree sparrows Passer montanus. Ibis 144: E67–E72.

COSEWIC. 2010. COSEWIC assessment and status report on the Acadian flycatcher *Empidonax virescens* in Canada. Committee on the Status of COSEWIC. Committee on the status of endangered wildlife in Canada, Ottawa. 38p

Currie D, Krupa AP, Burke T, Thompson DB. 1999. The effect of experimental male removals on extrapair paternity in the wheatear, *Oenanthe oenanthe*. Animal Behaviour 57: 145–152.

Derrickson KC, Breitwisch R. 1992. Mockingbird (*Mimus polyglottos*). In: Poole A, Stettenheim P, Gill F (eds). The birds of North America, no. 7. The Academy of Natural Sciences. Philadelphia, PA, USA. pp. 1–25.

Dhondt AA. 1987. Polygynous blue tits and monogamous great tits: does the polygyny-threshold model hold? The American Naturalist 129: 213–220.

Dickinson JL. 2001. Extrapair copulations in western bluebirds (*Sialia mexicana*): Female receptivity favors older males. Behavioral Ecology and Sociobiology 50: 423–429.

Dixon A, Ross D, O'Malley SLC, Burke T. 1994. Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. Nature 371: 698–700.

Duckworth RA. 2004. Condition-dependent sexual traits and social dominance in the house finch. Behavioral Ecology 15: 779–784.

Dunn PO, Cockburn A. 1998. Costs and benefits of extra-group paternity in superb fairy-wrens. In Parker PG, Burley NT (eds). Avian reproductive tactics: female and male perspectives. Washington DC, USA: The American Ornithologists Union. pp. 147–161.

Durrant KL, Hughes M. 2005. Differing rates of extra-group paternity between two populations of the Australian Magpie (*Gymnorhina tibicen*). Behavioral Ecology and Sociobiology 57: 536–545.

Dyrcz A Zdunek W. 1993. Breeding ecology of the aquatic warbler (*Acrocephalus paludicola*) in the Biebrza Marshes (NE Poland). Ibis 135: 181–189.

Ewen JG, Ciborowski KL, Clarke RH, Boulton RL, Clarke MF. 2008. Evidence of extra-pair paternity in two socially monogamous Australian passerines: the Crescent Honeyeater and the Yellow-faced Honeyeater. Emu 108: 133–137.

Fornasari L, Bottoni L, Sacchi N, Massa R. 1994. Home range overlapping and socio-sexual relationships in the red-backed shrike *Lanius collurio*. Ethology Ecology and Evolution 6: 169–177.

Forstmeier W. 2003. Extra-pair paternity in the dusky warbler, *Phylloscopus fuscatus*: a test of the 'constrained female hypothesis'. Behaviour 140: 1117–1134.

Freeman-Gallant CR. 1997. Extra-pair paternity in monogamous and polygynous savannah sparrows, *Passerculus sandwichensis*. Animal Behaviour 53: 397–404.

Friedl TW, Klump GM. 2002. Extrapair paternity in the red bishop (*Euplectes orix*): is there evidence for the good-genes hypothesis? Behaviour 139: 777–800.

Garamszegi LZ, Møller AP, Török J, Michl G, Péczely P, Richard M. 2004. Immune challenge mediates vocal communication in a passerine bird: an experiment. Behavioral Ecology 15: 148–157.

Garvin JC, Abroe B, Pedersen MC, Dunn PO, Whittingham LA. 2006. Immune response of nestling warblers varies with extra-pair paternity and temperature. Molecular Ecology 15: 3833–3840.

Gissing GJ, Crease TJ, Middleton ALA, 1998. Extrapair paternity associated with renesting in the American goldfinch. Auk: 115: 230–234.

Green DJ, Peters A, Cockburn A. 2002. Extra-pair paternity and mate-guarding behaviour in the brown thornbill. Australian Journal of Zoology 50: 565–580.

Griffith SC, Owen IPF, Thurman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11: 2195–2212.

Gyllensten UB, Jakobsson S, Temrin H. 1990. No evidence of illegitimate young in monogamous and polygynous warblers. Nature 343: 168–170.

Hartley I, Shepherd M, Robson T, Burke T. 1993. Reproductive success of polygynous male corn buntings (*Miliaria calandra*) as confirmed by DNA fingerprinting. Behavioral Ecology, 4: 310 –317.

Hartley IR, Shepherd M, Thompson DBA. 1996. Habitat selection and polygyny in breeding corn buntings *Miliaria calandra*. Ibis 137: 508–514.

Higgins PJ, Peter JM, Cowling SJ. 2006. Handbook of Australian, New Zealand and Antarctic birds. Vol. 7, boatbill to starlings. Oxford University Press, Melbourne.

Hill GE, Montgomerie R, Roeder R, Boag P. 1994. Behavioral Ecology and Sociobiology. 35: 193–199.

Hill DP, Gould LK. 1997. Chestnut-collared longspur (*Calcarius ornatus*). In: Poole A (ed). The birds of North America, no. 288. The Academy of Natural Sciences, Philadelphia, PN, USA; Cornell Lab of Ornithology, Ithaca, NY, USA. Retrieved from: http://bna.birds.cornell.edu.libproxy.uregina.ca:2048/bna/species/288

Hill CE, Akçay C, Campbell E, Beecher MD. 2011. Extrapair paternity, song, and genetic quality in song sparrows. Behavioral Ecology 22: 73–81.

Hoffman JI, Munro K, Kilner R, Amos W. 2010. High rates of infidelity in the Grey Fantail *Rhipidura albiscapa* suggest that testis size may be a better correlate of extra-pair paternity than sexual dimorphism. Ibis 152: 378–385.

Hoi H, Hoi-Leitner M. 1997. An alternative route to coloniality in the Bearded Tit: females pursue extra-pair fertilizations. Behavioral Ecology 8: 113–119.

Hope D. 1990. Brood feeding and division of parental care by crested tits. Scottish Birds, 16, 19–24.

Hurd PL, Weatherhead PJ, McRae SB. 1991. Parental consumption of nestling feces: good food or sound economics? Behavioral Ecology, 2, 69–76.

Ille R, Hoi H. 2001. The role of simple song in the marsh warbler (*Acrocephalus palustris*). In: Hoi H (ed). The ecology of reed birds. Osterreichische Akademie der Wissenschaften, Vienna, Austria. pp. 111–120.

Johnson LS, Thompson CF, Sakaluk SK, Neuhäuser M, Johnson BGP, Soukup SS, Forsythe SJ, Masters BS. 2009. Extra-pair young in house wren broods are more likely to be male than female. Proceedings of the Royal Society of London B: Biological Sciences 276: 2285–2289.

Kempenaers B, Everding S, Bishop C, Robertson RJ. 2001. Extra-pair Paternity and the reproductive role of male flaters in the tree swallow (*Tachycineta Bicolor*). Behavioral Ecology & Sociobiology 49: 251–259.

Krokene C, Anthonisen K, Lifjeld JT, Amundsen T. 1996. Paternity and paternity assurance behaviour in the bluethroat, *Luscinia s. svecica*. Animal Behaviour 52: 405–417.

Kulaszewicz I, Wojczulanis-Jakubas K, Jakubas D. 2015. Variation of the Savi's warbler (*Locustella luscinioides*) leucocyte profiles and body condition in relation to age, sex and moult. Annales Zoologici Fennici 52: 325–338.

Laubach ZM, Perng W, Lombardo M, Murdock C, Foufoupouls J. 2015. Determinants of parental care in mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*). Auk 132: 893–902.

Leech DI, Hartley IR, Stewart IRK, Griffith SC, Burke T. 2001. No effect of parental quality or extra-pair paternity on brood sex ratio in the blue tit (*Parus caeruleus*). Behavioral Ecology 12: 674–680.

Leisler B, Wink M. 2000. Frequencies of multiple paternity in three Acrocephalus species (Aves Sylviidae) with different mating systems (*A. palustris, A. arundinaceus, A. paludicola*). Ethology Ecology and Evolution 12: 237–249.

Lens L, Van Dongen S, Van den Broeck M, Van Broeckhoven C, Dhondt AA. 1997. Why female crested tits copulate repeatedly with the same partner: evidence for the mate assessment hypothesis. Behavioral Ecology 8: 87–91.

Logan CA. 1991. Mate switching and mate choice in female Northern mockingbirds: facultative monogamy. The Wilson Bulletin 103: 277–281.

Lowther PE. 1975. Geographic and geological variation in the family Icteridae. Wilson Bulletin 87: 481–495.

Lozano GA, Lemon RE. 1995. Food abundance and parental care in yellow warblers (*Dendroica petechia*). Behavioral Ecology and Sociobiology 37: 45–50.

Lynn SE, Wingfield JC. 2003. Male chestnut-collared longspurs are essential for nestling survival: A removal study. Hormones and Behavior 47: 170–177.

Margraf N, Cockburn A. 2013. Helping behaviour and parental care in fairy-wrens (Malurus). Emu 113: 294–301.

Møller AP, de Lope F, Saino N. 1995. Sexual selection in the barn swallow *Hirundo rustica*. VI. Aerodynamic adaptations. Journal of Evolutionary Biology 8: 671–687.

Møller AP, Tegelström H. 1997. Extra-pair paternity and tail ornamentation in the barn swallow *Hirundo rustica*. Behavioral Ecology and Sociobiology 41: 353–360.

Moreno J. 1984. Parental care of fledged young, division of labor, and the development of foraging techniques in the northern wheatear (*Oenanthe oenanthe*). Auk 101: 741–758.

Morton ES, Stutchbury BJM, Howlett JS, Piper WH. 1998. Genetic monogamy in blue-headed vireos and a comparison with a sympatric vireo with extra-pair paternity. Behavioral Ecology 9: 515–524.

Moskwik MP, O'Connell MA. 2006. Male and female reproductive strategies in the polygynous bobolink. Northwest Science 80: 108–115.

Muller RE, Smith DG. 1978. Parent-offspring interactions in zebra finches. Auk 95: 485–495.

Murphy TG, Rosenthal MF, Montgomerie R, Tarvin KA. 2009. Female American goldfinches use carotenoid-based bill coloration to signal status. Behavioral Ecology, 20, 1348–1355.

Neto JM, Hansson B, Hasselquist, D. 2010. Low frequency of extra-pair paternity in Savi’s Warblers (*Locustella luscinioides*). Behaviour 147: 1413 –1429.

Norris KJ. 1990. Female choice and the quality of parental care in the great tit *Parus major*. Behavioral Ecology and Sociobiology 27: 275–281.

Orell M, Rytkönen S, Launonen V, Welling P, Koivula K, Kumpulainen K, Bachmann L. 1997. Low frequency extra-pair paternity in the willow tit *Parus montanus* as revealed by DNA fingerprinting. Ibis 139: 562–566.

Otter K, Ratcliffe L, Michaud D, Boag PT. 1998. Do female chickadees prefer high-ranking males as extra-pair partners? Behavioral Ecology and Sociobiology 43: 25–36.

Otter KA Stewart IRK, McGregor PK, Terry AMR, Dabelsteen T, Burke T. 2001. Extra-pair paternity among great tits *Parus major* following manipulation of male signals. Journal of Animal Behaviour 32: 338–344.

Pärt T, Gustafsson L. 1989. Breeding dispersal in the collared flycatcher (*Ficedula albicollis*): possible causes and reproductive consequences. Journal of Animal Ecology 58: 305–320.

Pinowski J, Pinowska B, Romanowski VR. 2015. Post-breeding aggressive territorial behaviour in Eurasian tree sparrows (*Passer montanus*): territory or mate defence? Tichodroma 27: 2–10.

Piper WH, Slater G. 1993. Polyandry and incest avoidance in the cooperative stripe-backed wren of Venezuela. Behaviour 124: 227–247.

Pitcher TE, Stutchbury BJ. 2000. Extraterritorial forays and male parental care in hooded warblers. Animal Behaviour 59: 1261–1269.

Powell GVN, Jones LH. 1978. An observation of polygyny in the common yellowthroat. Wilson Bulletin 90: 656–657.

Pribil S, Picman J. 1996. Polygyny in the red-winged blackbird: do females prefer monogamy or polygamy? Behavioral Ecology and Sociobiology 38: 183–190.

Qvarnström A, Pärt T, Sheldon BC. 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. Nature 405: 344–347.

Rabenold PP, Rabenold KN, Piper WH, Decker MD, Haydock J, Zack SW. 1990. Shared paternity revealed by genetic analysis in cooperatively breeding tropical wrens. Nature 326: 392–394.

Reyer H-U., Bollmann K, Schläpfer AR, Schymainda A, Klecack G. 1997. Ecological determinants of extrapair fertilizations and egg dumping in alpine water pipits (Anthus spinoletta). Behavioral Ecology 8: 534–543.

Richardson DS, Burke T. 1999. Extra-pair paternity in relation to male age in Bullock's orioles. Molecular Ecology 8: 2115–2126.

Riley HT, Bryant DM, Carter RE, Parkin DT. 1995. Extra-pair fertilizations and paternity defence in house martins, *Delichon urbica*. Animal Behaviour 49: 495–509.

Rowe KMC, Weatherhead PJ. 2007. Social and ecological factors affecting paternity allocation in American robins with overlapping broods. Behavioral Ecology and Sociobiology. 61: 1283–1291.

Rytkönen S, Orell M, Koivula K, Soppela M. 1995. Correlation between two components of parental investment: nest defence intensity and nestling provisioning effort of willow tits. Oecologia 104: 386–393.

Ritchison G, Klatt PH, Westneat DF. 1994. Mate-guarding and extrapair paternity in northern cardinals. Condor 96: 1055–1063.

Saino N, Primmer CR, Ellegren H, Møller AP. 1997. An experimental study of paternity and tail ornamentation in the barn swallow (*Hirundo rustica*). Evolution 51: 562–570.

Sandell MI, Smith HG, Bruun M. 1996. Paternal care in the European starling, *Sturnus vulgaris*: nestling provisioning. Behavioral Ecology and Sociobiology 39: 301–309.

Schmoll T, Schurr FM, Winkel W, Epplen JT, Lubjuhn T. 2009. Lifespan, lifetime reproductive performance and paternity loss of within-pair and extra-pair offspring in the coal tit *Periparus ater*. Proceedings of the Royal Society of London B: Biological Sciences 276: 337–345.

Schwarzová L, Šimek J, Coppack T, Tryjanowski P. 2008. Male-biased sex of extra pair young in the socially monogamous red-backed shrike *Lanius collurio*. Acta Ornithologica 43: 235–239.

Schulze-Hagen K, Swatschek I., Dyrcz A, Wink M. 1993. Journal of Ornithology 134: 145–154. Scriba, M.F., Goyman, W. (2010). European robins (*Erithacus rubecula*) lack an increase in testosterone during simulated territorial intrusions. Journal of Ornithology 151: 607–614.

Sejberg D, Bensch D, Hasselquist D. 2000. Nestling provisioning in polygynous great reed warblers (*Acrocephalus arundinaceus*): do males bring larger prey to compensate for fewer nest visits? Behavioral Ecology and Sociobiology 47: 213–219.

Seutin G, Boag PT, White BN, Ratcliffe LM. 1991. Sequential polyandry in the common redpoll (*Carduelis flammea*). Auk 108: 166–198.

Sheldon BC, Burke T. 1994. Copulation behavior and paternity in the chaffinch. Behavioral Ecology and Sociobiology 34: 149–156.

Sheldon BC, Ellegren H. 1999. Sexual selection resulting from extrapair paternity in collared flycatchers. Animal Behaviour 57: 285–298.

Sherman PW, Morton ML. 1988. Extra-pair fertilizations in mountain white-crowned sparrows. Behavioural Ecology and Sociobiology 22: 413–420.

Smiseth PT, Örnborg J, Andersson S, Amundsen T. 2001. Is male plumage reflectance correlated with paternal care in bluethroats? Behavioral Ecology 12: 164–170.

Smith HG, von Schantz T. 1993. Extra-pair paternity in the European starling: the effect of polygyny. Condor 95: 1006–1015.

Soukup SS, Thompson CF. 1997. Social mating system affects the frequency of extra-pair paternity in house wrens. Animal Behaviour 54: 1089–1105.

Stewart IRK, Hanschu RD, Burke T, Westneat DF. 2006. Tests of ecological, phenotypic, and genetic correlates of extra-pair paternity in the House Sparrow. Condor 108: 399–413.

Stodola KW, Linder ET, Buehler DA, Franzreb KE, Cooper RJ. 2009. Parental care in the multi-brooded black-throated blue warbler. Condor 111: 497–502.

Stutchbury BJ, Rhymer JM, Morton ES. 1994. Extrapair paternity in hooded warblers. Behavioral Ecology 5: 384–392.

Sundberg J, Dixon A. 1996. Old, colourful male yellowhammers, *Emberiza citrinella*, benefit from extra-pair copulations. Animal Behaviour 52: 113–122.

Tobias JA. 1996. Mechanisms and functions of territoriality in the European robin *Erithacus rubecula* [PhD]. University of Cambridge, Cambridge, UK.

Tuttle EM. 1993. Mate choice and stable polymorphism in the white-throated sparrow [PhD]. State University of New York at Albany, Albany, NY, USA.

Tuttle EM. 2003. Alternative reproductive strategies in the white-throated sparrow: behavioral and genetic evidence. Behavioral Ecology 14: 425–432.

Van Roo BL, Ketterson ED, Sharp PJ. 2003. Testosterone and prolactin in two songbirds that differ in paternal care: the blue-headed vireo and the red-eyed vireo. Hormones and Behavior 44: 435–431.

Veiga JP. 1992. Why are house sparrows predominantly monogamous: a test of hypotheses. Animal Behaviour 43: 361–370.

von Haartman L. 1951. Successive polygamy. Behavior 3: 256–274.

Wagner RH, Schug MD, Morton ES. 1996a. Condition-dependent control of paternity by female purple martins: implications of coloniality. Behavioral Ecology and Sociobiology 38: 379–389.

Wagner RH, Schug MD, Morton ES. 1996b. Confidence of paternity, actual paternity and parental effort by purple martins. Animal Behaviour 52: 123–132.

Webster MS, Chuang-Dobbs HC, Holmes RT. 2001. Microsatellite identification of extrapair sires in a socially monogamous warbler. Behavioral Ecology 12: 439–446.

Webster MS, Tarvin KA, Tuttle EM, Pruett-Jones S. 2004. Reproductive promiscuity in the splendid fairywren: effects of group size and auxiliary reproduction. Behavioral Ecology 15: 907–915.

Westneat DF. 1987. Extra-pair fertilizations in a predominantly monogamous bird: genetic evidence. Animal Behaviour 35: 877–886.

Westneat DF. 1988. Male parental care and extrapair copulations in the indigo bunting. Auk 105: 149–160.

Westneat DF. 1993. Polygyny and extrapair fertilizations in eastern red-winged blackbirds (*Agelaius phoeniceus*). Behavioral Ecology 4: 49–60.

Wheelwright NT, Schultz CB, Hodum PJ. 1992. Polygyny and male parental care in savannah sparrows: Effects on female fitness. Behavioral Ecology and Sociobiology 31: 279–289.

Whittingham LA, Lifjeld JT. 1995. Extra-pair fertilizations increase the opportunity for sexual selection in the monogamous House Martin *Delichon urbica*. Journal of Avian Biology 26: 283–288.

Wolf L, Ketterson ED, Nolan Jr V. 1988. Paternal influence on growth and survival of dark-eyed junco young: do parental males benefit? Animal Behaviour 36: 1601–1618.

Woolfenden BE, Stutchbury BM, Morton ES. 2005. Male Acadian Flycatchers, *Empidonax virescens*, obtain extrapair fertilizations with distant females. Animal Behaviour 69: 921–929.

Yezerinac SM, Weatherhead PJ. 1997. Extra-pair mating, male plumage coloration and sexual selection in yellow warblers (*Dendroica petechia*). Proceedings of the Royal Society of London B: Biological Sciences 264: 527–532.

Zilberman R, Moav B, Yom-Tov Y. 1999. Extra-pair paternity in the socially monogamous orange-tufted sunbird (*Nectarinia osea osea*). Israel Journal of Zoology 45: 407–421.

Appendix 3: Reprints of other papers published or in press during the PhD study

ORIGINAL ARTICLE

So much for the city: urban-rural song variation in a widespread Asiatic songbird

Running Head: OMR urban-rural song variation

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Abstract

Song plays a fundamental role in intraspecific communication in songbirds. The temporal and structural components of songs can vary in different habitats. These include urban habitats where anthropogenic sounds and alteration of habitat structure can significantly affect songbird vocal behavior. Urban-rural variations in song complexity, song length and syllable rate are not fully understood. In this study, using the oriental magpie-robin (*Copsychus saularis*) as a model, we investigated urban-rural variation in song complexity, song length, syllable rate, syllable length and inter-syllable interval. Comparing urban and rural songs from 7 countries across its natural Asiatic range (Bangladesh, India, Malaysia, Nepal, Singapore, Sri Lanka and Thailand), we found no significant differences in oriental magpie-robin song complexity. However, we found significant differences in temporal song variables between urban and rural sites. Longer songs and inter-syllable intervals in addition to slower syllable rates within urban sites contributed the most to this variance. This indicates that the urban environment may have driven production of longer and slower songs to maximize efficient transmission of important song information in urban habitats.

INTRODUCTION

Song plays a fundamental role in mate selection and territory defence in songbirds (Kroodsma & Miller 1996; Catchpole & Slater 2008). The acoustic adaptation hypothesis suggests some species adjust vocal signals to minimize sound degradation during propagation within different habitats, thereby affecting song characteristics (Boncoraglio & Saino 2007; Hill *et al*. 2013; Smith *et al*. 2013). For example, songs in open habitats are shorter than in forested areas (Handford & Lougheed 1991), furthermore in closed habitats there is lower note repetition, fewer frequency modulations, and narrower bandwidths (Tubaro & Segura 1995; Brumm & Naguib 2009; Ey & Fischer 2009; Tobias *et al*. 2010).

Rapid adaptations to extensive habitat changes and increased noise levels that often characterize progressing urbanization increase songbird survival and breeding success in urban habitats (Dominoni *et al*. 2013; Dowling *et al*. 2013). Urbanization can affect songbird vocal communication by introducing structural changes to habitat and anthropogenic noise which alters the acoustic properties of the environment, in turn masking acoustic avian signals (Kight & Swaddle 2015). Investigations on the effect of urbanization on song have focused heavily on song frequency changes (Wood & Yezerinac 2006; Seger *et al*. 2010; Dowling *et al*. 2013; Slabbekoorn 2013; Swaddle *et al*. 2015; Derryberry *et al*. 2016; Narango & Rodewald 2016; Roca *et al*. 2016). For example, the dominant frequency (the frequency that has the highest amplitude) of the second 'bee' note of mountain chickadee (*Poecile gambeli*) song was higher-pitched in urban individuals (Lazerte *et al*. 2017). In addition, upward minimum frequency shifts have been reported to occur in the presence of urban noise (Nemeth *et al*. 2013). These vocal alterations function partly to reduce signal masking by ambient low frequency anthropogenic noise such as rush-hour traffic (Warren *et al*. 2006; Dowling *et al*. 2013). However, evidence suggests that spectrogram analysis may be an unreliable method for measuring frequencies in noisy areas (Grace & Anderson 2015). The associated amplitude increases make visible, previously unseen softer renditions of the same spectral information, which may lead to frequency measurement errors (Zollinger *et al*. 2012).

One sexually-selected component of bird song is song complexity (Ballentine *et al*. 2003). There is evidence that complex songs are energetically and physiologically-costly signals (Nottebohm *et al*. 1981; Otter *et al*. 1997). Complex songs may function as 'honest signals' indicating male quality (Spencer *et al*. 2003). Therefore, song complexity in songbirds is critical in mate choice and reproductive fitness (Briefer *et al*. 2010). The song complexity of an individual can be reflected in repertoire size, number of fundamental sound units (syllables), and the number of transitions from 1 syllable type to another (Boogert *et al*. 2008; Sasahara *et al*. 2012). Although syllable transition quantification can also be considered as a variable measuring song structure (Honda & Okanoya 1999).

The plasticity of a behavioral trait can potentially allow an individual to adapt rapidly and reversibly to environmental changes (Duckworth & Kruuk 2009) such as habitat modifications. However, plasticity integration, or correlations of component plasticity (see Schlichting 1989), can lead to the reduction in sexually-selected trait elaboration such as song complexity in terms of syllable diversity (Montague *et al*. 2013). Aside from syllable diversity however, little is known how urban noise and infrastructure affect other song complexity parameters such as the number of syllables per song and the number of syllable transitions. Such information is important for understanding the behavioral adaptation of songbirds to modified habitats.

Another important aspect of avian song is song length (Sakata *et al*. 2008), measured from the start of the first note to the end of the last note or syllable of a song. Song length may indicate a male's energy reserves, therefore is a key song component in female mate choice (Nolan & Hill 2004). Song length in males is also important in a territorial context. However, findings in studies examining effects of song length on rival response are inconsistent. Nelson & Poesel (2010) found shorter songs elicit more aggressive responses, while Linhart *et al*. (2012) showed that longer songs can stimulate more aggressive responses from rival males. Research examining the effect of urbanization on song length has also yielded mixed findings. For example, song length was not significantly affected by urbanization in silvereyes (*Zosterops lateralis*, Potvin *et al*. 2011), while songs in noisy urban areas were shorter than in rural areas in red-winged blackbirds (*Agelaius phoeniceus*, Ríos-Chelén *et al*. 2015) and house finches (*Carpodacus mexicanus*, Fernández-Juricic *et al*. 2005). Yet songs were longer in noisy urban areas than in quieter rural areas in vermilion flycatchers (*Pyrocephalus rubinus*) (Ríos-Chelén *et al*. 2013), and great tits (*Parus major*, Hamao *et al*. 2011). One possibility for increased song length in urban areas is that tall urban buildings cause sound reverberation and consequently syllable degradation. Birds may subsequently alter or adapt vocal communication patterns by producing longer intervals between syllables to reduce sound reverberation thereby maximising efficient sound transmission. This in turn will increase the length of songs (Potvin *et al.* 2011). Syllable rate (the number of syllables per second in each song), is another song trait related to male songbird aggression and male quality (Podos 1996; Linhart *et al*. 2013; Funghi *et al*. 2015). Syllable rate was found to be lower in urban areas (Potvin *et al*. 2011) which is likely to be associated with longer inter-syllable intervals observed in urban areas.

In this study, we investigated urban-rural variation of both song complexity and temporal song variables in a vocally complex Asiatic songbird, the oriental magpie-robin ('OMR' from herein, *Copsychus saularis*). OMR are small omnivorous songbirds in the Old World flycatcher family (Muscicapidae) with a natural geographic distribution spanning South and South-East Asia (Sheldon *et al*. 2009). Males have highly varied and complex songs used for territory establishment (Bhatt *et al* 2000; Kumar & Bhatt 2001) and their song exhibits some geographical dialectal variations (Dunmak & Sitasuwan 2007). Females sing short, low amplitude songs in the presence of males (Kumar & Bhatt 2002). A study on a Nepalese OMR population suggested songs are comprized of between 5 to 10 syllables displayed in a frequency range spanning 2.5 to 6 kHz. The mean OMR syllable repertoire size is 7.4 ranging from 5 to 10. Individuals have a maximum of 2 songs within their repertoire meaning much repetition of the same song (Bhattacharya *et al*. 2007). OMR are common in both urban and natural areas (Prakash & Manasvili 2013) which makes this species a good model for studying the effect of urbanization on songbird vocal behavior.

We compared OMR song complexity (measured by the number of syllables per song, syllable diversity and syllable transitions) in addition to temporal variables (inter-syllable interval, song and syllable length in addition to syllable rate) between urban and rural areas in 7 different countries within OMR's natural range (Bangladesh, India, Malaysia, Nepal, Singapore, Sri Lanka and Thailand). We predicted song complexity would be lower in urban areas and songs longer due to plasticity integration and to reduce sound degradation respectively. We also predicted that increased song length would be combined with slower syllable rate and longer inter-syllable in urban areas to help reduce reverberation as certain large man-made structures can cause sound degradation. We also predicted longer syllable length which could increase the probability of signal detection in noise, as longer signals are generally easier to detect in noise (Pohl *et al*. 2013). OMR songs were analyzed in different cities and nearby natural areas within Asia to test whether urbanization had a common effect on song characteristics. Studies examining urban-rural song variations in mainland Asia have been scarce, therefore this study provides important baseline data contributing to avian behavioral adaptations to urban habitats in this geographic region.

MATERIAL AND METHODS

Data collection

Song data were obtained from both the wild and online resources. Data were attained from both urban and rural areas in each country where possible. Recordings of male OMR territorial songs from Bangladesh, India, Malaysia, Nepal, Singapore (urban only), Sri Lanka, and Thailand were extracted from the Cornell Lab of Ornithology's Macaulay Library archive of wildlife sounds and videos (Cornell University; www.macaulaylibrary.org), the Xeno-Canto citizen science project website (www.xeno-canto.org), and Michigan State University's Project AvoCet (www.avocet.zoology.msu.edu) (Table 1). In South-East Asia, the OMR breeding season spans January to June (Dunmak & Sitasuwan 2007). Recordings obtained online from this area were conducted during this period where recording date was available. OMR breeding season in India commences from March to August (Bhatt *et al*. 2014). In Sri Lanka, breeding proceeds year-round but is the least active in October (Clement & Rose 2015) and recordings from here were made in March. Supplementary to the online collection of recordings, field recordings from male OMR were obtained from 3 locations in Nepal: Sauraha, Chitwan (urban); Hotel Billabong, Barahi Tole, Lakeside, Pokhara (urban); and Khudi, Lamjung District, Gandaki Zone (rural). A Marantz PMD620 solid-state digital recorder (Marantz, Kanagawa, Japan), paired with a Sennheiser ME67 shotgun long-range directional microphone (Sennheiser, Old Lyme, CT) were used to obtain these recordings from Nepal. These recordings were conducted immediately prior to breeding season in mid-February 2014 when males establish and defend territories. Other OMR recordings from Nepal were conducted during breeding season, March to August (Kumar & Bhatt, 2002; Bhattacharya *et al*. 2007; Van Riessen 2011). In total, all but 2 recordings used within this study (16/18) were made before 1430 h, where time of recording is known.

Only songs from high quality recordings with good signal-to-noise ratios (≥10 db) were used for analysis (all 3 of the above archives have stringent quality ratings for each recording, and only recordings with the top 2 quality ratings were used in the analysis). There was generally only 1 recording available from each exact location, including the field recordings conducted in Nepal, except for Singapore Botanical Gardens and Teman Nagara National Park, Pahang, Malaysia, where 2 were available. In both cases, each separate recording was from a different individual, identified by the recordist as such (the recordist was the same at both locations). Available OMR recordings were limited but were chosen at random from each country where there were enough to facilitate randomness and so long as they did not violate the signal-to-noise and quality rating criteria mentioned above. For each male OMR song, information on location (geographical coordinates), habitat, behavioral context, recording date, recording time (except for 4 recordings of which time was not recorded), distance from bird, and recording equipment used were also extracted from the database. The same data was also noted for the Nepal field recordings. Each sound file was considered to be from the same individual unless stated on the recording. As a general rule, each OMR song was followed by a natural extended interval of silence (of approximately 2.5–3 s), therefore we considered that a new song had started when a new series of syllables began, following this period of silence (Fig. 1).

Urban/rural habitat selection criteria

A song was considered to originate from an urban area if the recording location was within the confines of a densely built-up area, and the recording was coupled with anthropogenic noise such as traffic sound. Nevertheless, if urban noise overwhelmed the recording, the song would not be analysed as per the signal-to-noise ratio criteria. It was considered rural if the recording location predominantly consisted of pasture and/or forest land (i.e. within a rural reserve, national or forest park), with no nearby densely built-up areas nor anthropogenic noise. Google Maps (Mountain View, California) was used to confirm the urban or rural status of each recording using location information given in the recording.

Data extraction

All 238 recordings were sampled at 44100 Hz at a resolution of 24-bits. Audio files from the Macaulay Library and AvoCet, and field recordings from Nepal, were all written and recorded as lossless uncompressed wav files. The 6 recordings taken from the online audio archive Xeno-Canto were mp3 files which were converted to wav files. The analytical software (Raven Pro 1.4 Beta Version, Cornell Lab of Ornithology, Ithaca, NY, USA) only reads wav files. The sampling rate was changed to 44100 Hz, and bit resolution changed to 24-bits using Online Convert (www.online-convert.com). The Xeno-Canto archive has been used to provide compressed sound files in other studies analysing and examining avian song structure (e.g. Weir & Wheatcroft 2011; Greig *et al*. 2013) without having any significant impact on song structural integrity. There is evidence that file compression has no effect on sound quality (Rempel *et al*. 2005). However, it is suggested that song structural features such as minimum and maximum frequencies can be affected by file compression of bird recordings (Stowell & Plumbley 2014) although these were not measured in this study. We acknowledge that the recording quality on these files may have been marginally lower than for the other sound samples used in this study. We are however confident that this had a negligible effect on the variables employed within our study.

The song spectrograms were digitized and parameters measured using Raven Pro. For all recordings, spectrograms were created by Discrete Fourier Transform (DFT) with a Hann window. The frame length was set at 256 points. Additionally, a 50% frame overlap with hop size of 2.9 ms was used. Frequency grid spacing of 172 Hz was also employed and the bandwidth was set at 3 dB. To investigate the variation in song complexity and temporal song parameters of male OMR between urban and rural locations, we used Raven Pro to extract 3 song complexity variables: number of syllables, syllable diversity, and syllable transitions; and 4 temporal parameters: inter-syllable interval, song length, syllable length, and syllable rate (Fig. 2; refer to Table 2 for definitions).

Statistical analysis

After normalizing all song variables, we used a non-metric multidimensional scaling plot (NMDS) (using a Euclidean resemblance measure) to provide a visual summary of the patterns of Euclidean values among the samples. Since no direct inferences were made using the NMDS, we plotted all songs (labeled by bird). However, given that each bird sang multiple songs, we averaged all variables for each individual bird (i.e. after averaging, the song data consisted of 23 rows which were considered independent datapoints).

Using the averaged data, permutational analysis of variance (PERMANOVA) was used to test the differences in song variables between urban and rural areas, with 'site' as a fixed factor (urban or rural) and 'country' a random factor controlling for effects on song across countries which could confound any variance observed. We ran 1 PERMANOVA test for song complexity and 1 for temporal variables using the PERMANOVA+ add-on package for PRIMER (Anderson *et al*. 2001). The PERMANOVA resemblance matrices were based on Euclidean distances.

We also used the averaged data to run 2 canonical analysis of principal coordinates tests (CAP, Anderson & Willis 2003): 1 for song complexity variables and 1 for the temporal variables to determine whether the song variables were sufficient to discriminate between songs from urban and rural habitats. Leave-one-out cross-validation was used to assess the discriminatory power of the CAP model (Stone 1974). All multivariate hypothesis tests were run in PRIMER v.6 (Clarke & Gorley 2006).

RESULTS

Firstly, PERMANOVA tests suggested there were no evidence of any differences in OMR song complexity (*Pseudo* $F_{19,23} = 0.6807$; $P = 0.654$; 999 permutations) or temporal song variables (*Pseudo* $F_{18,23} = 1.4733$; $P = 0.223$; 999 permutations) between countries.

A PERMANOVA test also revealed there was no evidence of a difference in song complexity between urban and rural areas (*Pseudo* $F_{19,23} = 0.9235$; $P = 0.365$; 999 permutations, Table 3). A CAP analysis showed 13/23 songs (56.52%) were correctly assigned to urban or rural songs.

There were however significant differences in temporal song variables between urban and rural areas in OMR (*Pseudo* $F_{18,23} = 3.4014$; $P = 0.026$; 999 permutations, Table 3). A CAP analysis showed 17/23 songs (72.73%) were correctly assigned to urban or rural songs. The NMDS (Fig. 3) indicated that the difference in the positions of urban and rural songs was primarily along the vertical axis. This axis was most strongly correlated with the 4 temporal variables (inter-syllable interval, song length, syllable length, syllable rate). Inter-syllable interval and song length were significantly longer in urban areas than rural areas. In contrast, the complexity variables correlate with the horizontal axis, which does not discriminate strongly between urban and rural songs.

DISCUSSION

We tested for song complexity variation between urban and rural habitats in an Asiatic songbird, OMR, using multiple song complexity measures. Our results suggest song complexity was not significantly different between urban and rural areas. Nonetheless, the general pattern of urban-rural disparity in components of song complexity appears to be consistent across other songbird species. For example, Eurasian blackbirds (*Turdus merula*) produced fewer notes per song in cities (Nemeth & Brumm 2009) and although urban silvereyes had similar syllable repertoires than rural individuals, they sang fewer syllable types per song (Potvin & Parris 2013). This is concordant with studies in the European robin where syllable diversity was markedly lower in urban areas due at least partly to increased plasticity integration influenced by the presence of background anthropogenic noise (Montague *et al*. 2013). Furthermore, in a cross-urban habitat analysis of tui (*Prosthemadera novaeseelandiae*) vocalizations, lower syllable diversity was discovered in those inhabiting areas situated closer to ambient motorway noise (Ludbrook 2015). We cannot rule out however that the lack of significant differences in OMR song complexity in our study may be partly explained by the presence of some site interactions. For example, in 2 countries (Bangladesh and Thailand), song complexity metrics such as syllable diversity were marginally higher in rural areas which is somewhat consistent with previous research mentioned above (Montague *et al*. 2013). Our study provides important baseline data for future studies with large sample sizes at each site, which would allow us to refine our understanding of the general effects of urbanization on song complexity. Future studies on collecting recordings with planned design, enabling the direct testing of hypotheses and full control of multiple environmental factors such as noise level, structure of environment and, for example, dialectal variations.

Contrary to song complexity, we found a significant difference between urban and rural areas for temporal song variables. Our results suggested that OMR songs in urban habitats were longer than those from rural habitats. The slower syllable rates found in urban areas in our study have also previously been reported for example in urban silvereyes (Potvin *et al*. 2011).

In our study, slower syllable rates were coupled with significantly longer inter-syllable intervals in urban areas. High syllable rates are energetically-expensive to maintain (Oberweger *et al*. 2001) therefore, individuals may reduce this energy cost and increase the effectiveness of their vocal communication by slowing songs down. This would help transmit critical information more effectively to conspecifics, such as the complexity of their syllable structures (Potvin *et al*. 2011). Evidence indicates that the physical characteristics of urban environments such as buildings affect the physics of vocal communication. Specifically, buildings are sound-reflective objects which distort and degrade song, causing repeated syllables to merge or be masked (Potvin *et al*. 2011). By lengthening inter-syllable intervals, songbirds maximize efficient sound transmission by reducing sound degradation (Potvin *et al*. 2011). These longer intervals are subsequently likely to give rise to longer songs. Our results provide further evidence suggesting that urbanization may affect vocal behavior in songbirds, not merely in terms of frequency shifts but in temporal song characteristics.

Intraspecific song divergence may be driven by marked differences in ambient noise levels between urban and rural environments. This may also be due to specific environmental or habitat characteristics (Ryan & Brenowitz 1985). For example, evidence suggests that urban environment structures such as tall buildings force birds into adapting to produce longer songs to combat environmental constraints on song transmission (Potvin *et al*. 2011). Large buildings may deflect and degrade song, causing syllables to overlap, become distorted, or be masked entirely (Potvin *et al*. 2011). Our study did not test whether temporal song characteristics are altered due to noise levels or urban buildings. Changes in song temporal patterns are likely to be due to a combination of both factors. Recent evidence suggests urban structures have strong and profound effects on both spectral and temporal vocal characteristics in some species (Job *et al*. 2016). For example, trills, which are rapidly repeated syllables, may be degraded due to the sound reflections from reflecting surfaces such as buildings (Naguib 2003). Other recent evidence suggests that increased anthropogenic noise reduces the effectivity of conspecific signaling, impairs signal discrimination (Kleist *et al*. 2016), and reduces song performance (Davidson *et al*. 2017). These factors may subsequently have detrimental long term effects on fitness and reproductive success in songbirds.

Longer songs in urban areas have been noted in previous studies in vermilion flycatchers (Ríos-Chelén *et al*. 2013). Increased song length may make songs easier to be heard by conspecifics (Pohl *et al*. 2013). Song length is an important signal in female mate choice due to its association with male energy reserves (Funghi *et al*. 2015). Longer songs therefore may be important signals advertising a male's ability to withstand the higher stress demands associated with urban inhabitation (Mikula 2014). Song length is also positively associated with the volume of the HVC, a telencephalic nucleus in the brain that controls song learning, the production of complex vocal signals and song perception in songbirds (Nottebohm 1999; Gil & Gahr 2002; Buchanan et al. 2004). It remains unclear however whether increased song length in urban OMR is a result of selection on the size of HVC or a plastic behavioral response to urban noise or a combination of factors. A recent study in zebra finches (*Taeniopygia guttata*) suggests exposure to strong traffic noise during development negatively impacts the volume of not only the HVC but also Area X, another region of the brain strongly associated with song learning (Potvin et al. 2016). Furthermore, there is evidence successful urban songbird species are more likely to have a large relative brain size and more likely to belong to large-brained taxonomic families (Maklakov et al. 2011). However, it remains unclear whether possessing a large brain has been the key to behavioral plasticity in songbirds successfully adapting to urban areas, though it is an area warranting extensive future efforts.

Evidence suggests conspecific density can also affect song behavior. For example, in the presence of a larger conspecific density, northern cardinals (*Cardinalis cardinalis*) sang longer and faster songs (Narango & Rodewald 2016). Furthermore, studies in great tits (*Parus major*) suggested that higher densities, thus intensified male-male competition, were associated with both increased minimum frequency and song phrase production (Hamao *et al*. 2011). These suggest density may be a driver of specific song feature production in such areas. Future studies examining urban-rural variation in songbird song complexity should therefore also consider conspecific density as a potential influence. This would provide a clearer picture as to localized contributory factors to song variation between urban and rural areas, rather than attributing these changes solely to urbanization. Understanding intraspecific song variation in different habitats can yield important insights into the evolution of avian communication in terms of acoustic adaptation (Ryan & Brenowitz 1985), cultural evolution (Luther & Baptista 2010) and dialectal variations (Lijtmaer & Tubaro 2007).

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REFERENCES

Anderson MJ (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**, 32–46.

Anderson MJ, Willis TJ (2003). Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* **84**, 511–25.

Ballentine B, Badyaev A, Hill GE (2003). Changes in song complexity correspond to periods of female fertility in blue grosbeaks (*Guira caerulea*). *Ethology* **109**, 55–66.

Bhatt D, Kumar A, Singh Y, Payne RB (2000). Territorial songs and calls in oriental magpie robin, *Copsychus saularis*. *Current Science* **78**, 722–28.

Bhatt D, Sethi VK, Kumar A, Singh A (2014). Some notes on the breeding behaviour of the oriental magpie robin (*Copsychus saularis*) from Uttarakhand, India. *Journal of Sustainability Science and Management* **9**, 112–19.

Bhattacharya SH, Cirillo J, Subba BR, Todt D (2007). Song performance rules in the oriental magpie-robin (*Copsychus saularis*). *Our Nature* **5**, 1–13.

Boncoraglio G, Saino N (2007). Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. *Functional Ecology* **21**, 134–42.

Boogert NJ, Giraldeau L-A, Lefbvre L (2008). Song complexity correlates with learning ability in zebra finch males. *Animal Behaviour* **76**, 1735–41.

Briefer E, Osiejuk TS, Rybak F, Aubin T (2010). Are bird song complexity and song sharing shaped by habitat structure? An information theory and statistical approach. *Journal of Theoretical Biology* **262**, 151–64.

Brumm H (2004). The impact of environmental noise on song amplitude in a territorial bird. *Journal of Animal Ecology* **73**, 434–40

Brumm H, Naguib M (2009). Environmental acoustics and the evolution of bird song, *Advances in the Study of Behaviour* **40**, 1–33.

Buchanan KL, Leitner S, Spencer KA, Goldsmith AR, Catchpole CK, 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. *Philosophical Transactions of the Royal Society B* **271**, 2381–86.

Cardoso GC, Mota PG (2007). Song diversification and complexity in canaries and seedeaters (Serinus spp.). *Biological Journal of the Linnean Society* **92**, 183–94

Catchpole CK (1980). Sexual selection and the evolution of complex songs among European warblers of the genus Acrocephalus. *Behaviour* **74**, 149–65.

Catchpole CK, Slater PJB (2008). *Bird song: biological themes and variations*. Cambridge University Press. New York.

Clarke KR, Gorley RN (2006). *Primer v6: user manual/tutorial*. PRIMER-E, Plymouth.

Clement P, Rose C (2015). *Robins and chats*. Bloomsbury, London.

Davidson BM, Antonova G, Dlott H, Barber JR, Francis CD (2017). Natural and anthropogenic sounds reduce song performance: insights from two emberizid species. *Behavioral Ecology* (In Press).

Derryberry EP, Danner RM, Danner JE, Derryberry GE, Phillips JN, Lipshutz SE, Gentry K, Luther DA (2016). Patterns of song across natural and anthropogenic soundscapes suggest that white-crowned sparrows minimize acoustic masking and maximize signal content. *PLOS ONE* **11(4)**, e0154456.

Dominoni DM, Helm B, Lehmann M, Dowse HB, Partecke J (2013). Clocks for the city: circadian differences between forest and city songbirds. *Philosophical Transactions of the Royal Society B* **280**, 20130593.

Dowling JL, Luther DA, Marra PP (2013). Comparative effects of urban development and anthropogenic noise on bird songs. *Behavioral Ecology* **23**, 201–09.

Duckworth RA, Kruuk LE (2009). Evolution of genetic integration between dispersal and colonization ability in a bird. *Evolution* **63**, 968–77.

Dunmak A, Sitasuwan N (2007). Song dialect of oriental magpie-robin (*Copsychus saularus*) in Northern Thailand. *Natural History Journal of Chulalongkorn University* **7**, 145–53.

Ey E, Fischer J (2009). The "acoustic adaptation hypothesis" - a review of the evidence from birds, anurans and mammals. *Bioacoustics* **19**, 21-48.

Fernández-Juricic E, Poston R, De Collibus K, Morgan T, Bastain B, Martin C, Jones K, Treminio T (2005) Microhabitat selection and singing behavior patterns of male house finches (*Carpodacus mexicanus*) in urban parks in a heavily urbanized landscape in the western U.S. *Urban Habitats* **3**, 49–69

Funghi S, Cardoso GC, Mota PG (2015). Increased syllable rate during aggressive singing in a bird with complex and fast song. *Journal of Avian Biology* **46**, 283–88.

Gil D, Gahr M (2002). The honesty of bird song: multiple constraints for multiple traits. *Trends in Ecology & Evolution* **17**, 133–41.

Grace M, Anderson RC (2015). No frequency shift in the "D" notes of Carolina chickadee calls in response to traffic noise. *Behavioral Ecology and Sociobiology* **69**, 253–63.

Greig EI, Price, JJ, Pruett-Jones S (2013). Song evolution in Maluridae: influences of natural and sexual selection on acoustic structure. *Emu* **113**, 270–81.

Hamao S, Watanabe M, Mori Y (2011). Urban noise and male density affect songs in the great tit *Parus major*. *Ethology, Ecology & Evolution* **23**, 111-19.

Handford P, Lougheed SC (1991). Variation in duration and frequency characters in the song of the rufous-collared sparrow, *Zonitrichia capensis*, with respecct to habitat, trill dialects and body size. *Condor* **93**, 644–58.

Hill SD, Ji W, Parker KA, Amiot C, Wells SJ (2013). A comparison of vocalisations between mainland tui (*Prosthemadera novaeseelandiae novaeseelandiae*) and Chatham Island tui (*P. n. chathamensis*). *New Zealand Journal of Ecology* **37**, 214–23.

Honda E, Okanoya K (1999). Acoustical and syntactical comparisons between songs of the white-backed munia (*Lonchura striata*) and its domesticated strain, the Bengalese finch (*Lonchura striata* var. *domestica*). *Zoological Science* **16**, 319–26.

Job JR, Kohler SL, Gill SA (2016). Song adjustments by an open habitat bird to anthropogenic noise, urban structure, and vegetation. *Behavioral Ecology* **27**. 1–11.

Kight CR, Swaddle JP (2015). Eastern bluebirds alter their song in response to anthropogenic changes in the acoustic environment. *Integrative Computational Biology* **55**, 418–31.

Kleist NJ, Guralnick RP, Cruz A, Francis CD (2016). Anthropogenic noise weakens territorial response to intruder's songs. *Ecosphere* 7, e01259.

Kroodsma DE, Miller EH (1996). *Ecology and evolution of acoustic communication in birds. Ecology and evolution of acoustic communication in birds*. Cornell University Press, Ithaca.

Kumar A (1999). *Characteristics and significance of calls, songs and visual displays in two avian species viz. Copsychus saularis and Pycnonotus cafer* (PhD thesis). Gurukul Kangri University, Haridwa.

Kumar A, Bhatt D (2002). Characteristics and significance of song in female oriental magpie-robin, *Copsychus saularis*. *Journal of Bombay Natural History Society* **99**, 54–8.

Lazerte SE, Otter KA, Slabbekoorn H (2017). Mountain chickadees adjust songs, calls and chorus composition with increasing ambient and experimental anthropogenic noise. *Urban Ecosystems* DOI 10.1007/s11252-017-0652-7

Linhart P, Jaška P, Petrusková T, Petrusek A, Fuchs R (2013). Being angry, singing fast? Signalling of aggressive motivation by syllable rate in a songbird with slow song. *Behavioural Processes* **100**, 139–45

Linhart P, Slabbekoorn H, Fuchs R (2012). The communicative significance of song frequency and song length in territorial chiffchaffs. *Behavioral Ecology* **23**, 1338–47.

Lijtmaer DA, Tubaro PL (2007). A reversed pattern of association between song dialects and habitat in the rufous-collared sparrow. *Condor* **109**, 658–67.

Ludbrook MR (2015). *Effects of urban noise on vocalisations of tūī* (*Prosthemadera novaeseelandiae*) (MSc thesis). Massey University, Auckland.

Luther D, Baptista L (2010). Urban noise and the cultural evolution of bird songs. *Philosophical Transactions of the Royal Society B* **277**, 469–73.

Maklakov AA, Immler S, Gonzalez-Voyer A, Rönn J. Kolm N (2011). Brains and the city: big-brained passerine birds succeed in urban environments. *Biology Letters* **7**, 730–32.

Mason NA, Shultz AJ, Burns KJ (2014). Elaborate visual and acoustic signals evolve independently in a large, phenotypically diverse radiation of songbirds. *Philosophical Transactions of the Royal Society B* **281**, 20140967.

Mikula P (2014). Pedestrian density influences flight distances of urban birds. *Ardea* **102**, 53–60.

Montague MJ, Danek-Gontard M, Kunc HP (2013). Phenotypic plasticity affects the response of a sexually selected trait to anthropogenic noise. *Behavioral Ecology* **24**, 342–48.

Naguib M (2003). Reverberation of rapid and slow trills: implications for signal adaptations to long-range communication. *Journal of the Acoustic Society of America* **113**, 1749–56.

Narango DL, Rodewald AD (2016). Urban-associated drivers of song variation along a rural–urban gradient. *Behavioral Ecology* **27**, 608–16.

Nelson DA, Poesel A (2011). Song length variation serves multiple functions in the white-crowned sparrow. *Behavioral Ecology and Sociobiology* **65**, 1103–11.

Nemeth E, Brumm H (2009). Blackbirds sing higher-pitched songs in cities: adaptation to habitat acoustics or side-effect of urbanisation. Animal Behaviour **78**, 637–41.

Nemeth E, Pieretti N, Zollinger SA, Geberzahn N, Partecke J et al., 2013. Bird song and anthropogenic noise: vocal constraints may explain why birds sing higher-frequency songs in cities. *Philosophical Transactions of the Royal Society B* **2080**, 1–7.

Nolan PM, Hill GE (2004). Female choice for song characteristics in the house finch. *Animal Behaviour* **67**, 403–10.

Nottebohm F (1981). A brain for all seasons: cyclic anatomical changes in song control nuclei of the canary brain. *Science* **214**, 1368–70.

Nottebohm F (1999). The design of animal communication. In: Hauser MD, Konishi M, eds. *The anatomy and timing of vocal learning in birds*. The MIT Press, Cambridge, MA, pp. 63–110.

Oberweger K, Goller F (2001). The metabolic cost of birdsong production. *Journal of Experimental Biology* **204**, 3379–85.

Otter K, Chruszcz B, Ratcliffe, L (1997). Honest advertisement and song output during the dawn chorus of black-capped chickadees. *Behavioral Ecology* **8**, 167–73.

Podos J (1996). Motor constraints on vocal development in a songbird. *Animal Behaviour* **51**, 1061–70.

Pohl NU, Slabbekoorn H, Neubauer H, Heil P, Klump GM, Langemann U (2013). Why longer song elements are easier to detect: threshold level-duration functions in the great tit and comparison with human data. *Journal of Comparative Physiology A* **199**, 239–52.

Potvin DA, Parris KM, Mulder RA (2011). Geographically pervasive effects of urban noise on frequency and syllable rate of songs and calls in silvereyes (*Zosterops lateralis*). *Philosophical Transactions of the Royal Society B* **278**, 2464–69.

Potvin DA, Parris KM (2013). Song convergence in multiple urban populations of silvereyes (*Zosterops lateralis*). *Ecology and Evolution* **2**, 1977–84.

Potvin DA, Mulder RA, Parris KM (2014). Acoustic, morphological, and genetic adaptations to urban habitats in the silvereye (*Zosterops lateralis*). In: Gil D, Brumm H, eds. *Avian Urban Ecology*. Oxford University Press, Oxford. pp. 171-80.

Potvin DA, Curcio MT, Swaddle JP, MacDougall-Shackleton SA (2016). Experimental exposure to urban and pink noise affects brain development and song learning in zebra finches (*Taenopygia guttata*). *Peer J* **4**, e2287.

Prakash S, Manasvini (2013). Urban avifaunal diversity: an indicator of anthropogenic pressures in Southern Ridge of Delhi. *Advances in Bioresearch* **4**, 135–44.

Rempel RS, Hobson KA, Holborn G, Van Wilgenburg SL, Elliott J (2005). Bioacoustic monitoring of forest songbirds: interpreter variability and effects of configuration and digital processing methods in the laboratory. *Journal of Field Ornithology* **76**, 1–11.

Ríos-Chelén AA, Quirós-Guerrero E, Gil D, Garcia CM (2013). Dealing with urban noise: vermilion flycatchers sing longer songs in noisier territories. *Behavioral Ecology and Sociobiology* **67**, 145–52.

Ríos-Chelén AA, Lee GC, Patricelli GL (2015). Anthropogenic noise is associated with changes in acoustic but not visual signals in red-winged blackbirds. *Behavioral Ecology and Sociobiology* **69**,1139–51.

Roca IT, Desrochers L, Giacomazzo M, Bertolo A, Bolduc P, Deschesnes R, Martin CA, Rainville V, Rheault G, Proulx R (2016). Shifting song frequencies in response to anthropogenic noise: a meta-analysis on birds and anurans *Behavioral Ecology* **27**, 1269–74.

Ryan MJ, Brenowitz EA (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. *American Naturalist* **126**, 87–100.

Sakata JT, Hampton CM, Brainard MS (2008). Social modulation of sequence and syllable variability in adult birdsong. *Journal of Neurophysiology* **99**, 1700–11.

Sasahara K, Cody ML, Cohen D, Taylor CE (2012). Structural design principles of complex bird songs: a network-based approach. *PLOS ONE* 7**(9)**, e44436.

Schlichting CD (1989). Phenotypic plasticity in Phlox.2. Plasticity of character correlations. *Acta Oecologica* **78**, 496–501.

Seger KD, Rodewald AD, Soha JA (2010). Urban noise predicts song frequency in Northern Cardinals and American Robins. *Bioacoustics* **20**, 267–76.

Sheldon FH, Lohman DJ, Lim HC, Zou F, Goodman SM Prawiradilaga DM, Winker K, Braile TM, Moyle RG (2009). Phylogeography of the magpie-robin species complex (Aves: Turdidae: *Copsychus*) reveals a Philippine species, an interesting isolating barrier and unusual dispersal patterns in the Indian Ocean and Southeast. *Asian Journal of Biogeography* **36**, 1070–83.

Slabbekoorn H (2013) Songs of the city: noise-dependent spectral plasticity in the acoustic phenotype of urban birds. *Animal Behaviour* **85**, 1089–1099.

Smith TB, Harrigan RJ, Kirschel ANG, Buermann W, Saatchi S, Blumstein DT, de Kort SR, Slabbekoorn H (2013). Predicting bird song from space. *Evolutionary Applications* **6**, 865–74.

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK (2003). Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Hormones and Behaviour* **44**, 132–39.

Stone M (1974). Cross-validatory choice and assessment of statistical predictions. *Journal of the Royal Statistical Society: Series B* (*Statistical Methodology*) **36**, 111–47.

Stowell D, Plumbley MD (2014). Large-scale analysis of frequency modulation in birdsong data bases. *Methods in Ecology and Evolution* **5**, 901–12.

Swaddle JP, Francis CD, Barber JR, Cooper CB, Kyba CCM, Dominoni DM, Shannon G, Aschehoug E, Goodwin SE, Kawahara AY, Luther D, Spoelstra K, Voss M, Longcore T (2015). A framework to assess evolutionary responses to anthropogenic light and sound. *Trends in Ecology & Evolution* **30**, 550–60.

Tobias JA, Aben J, Brumfield RT, Derryberry EP, Halfwek W, Slabbekoorn, Seddon N (2010). Song divergence by sensory drive in Amazonian birds. *Evolution* **64**, 2820–39.

Tubaro PL, Segura ET (1995). Geographic, ecological and subspecific variation in the song of the rufous-browed peppershrike (*Cyclarhis gujanensis*). *The Condor* **97**, 792–803.

Van Riessen A (2011). Territorial behaviour of the oriental magpie robin *Copsychus saularis* in rural Kathmandu. *Danphe Bird Conservation Nepal Newsletter* **20**, 1–4.

Warren PS, Katti M, Ermann M, Brazel A (2006). Urban bioacoustics: it's not just noise. *Animal Behaviour* **71**, 491-502.

Weir JT, Wheatcroft D (2011). A latitudinal gradient in rates of evolution of avian syllable diversity and song length. *Philosophical Transactions of the Royal Society B* **278**, 1713–20.

Wood WE, Yezerinac SM (2006). Song sparrow (*Melospiza melodia*) song varies with urban noise. *Auk* **123**, 650–59.

Zollinger SA, Podos J, Nemeth E, Goller F, Brumm H (2012). On the relationship between, and measurement of, amplitude and frequency in birdsong. *Animal Behaviour* **84**, e1–e9.

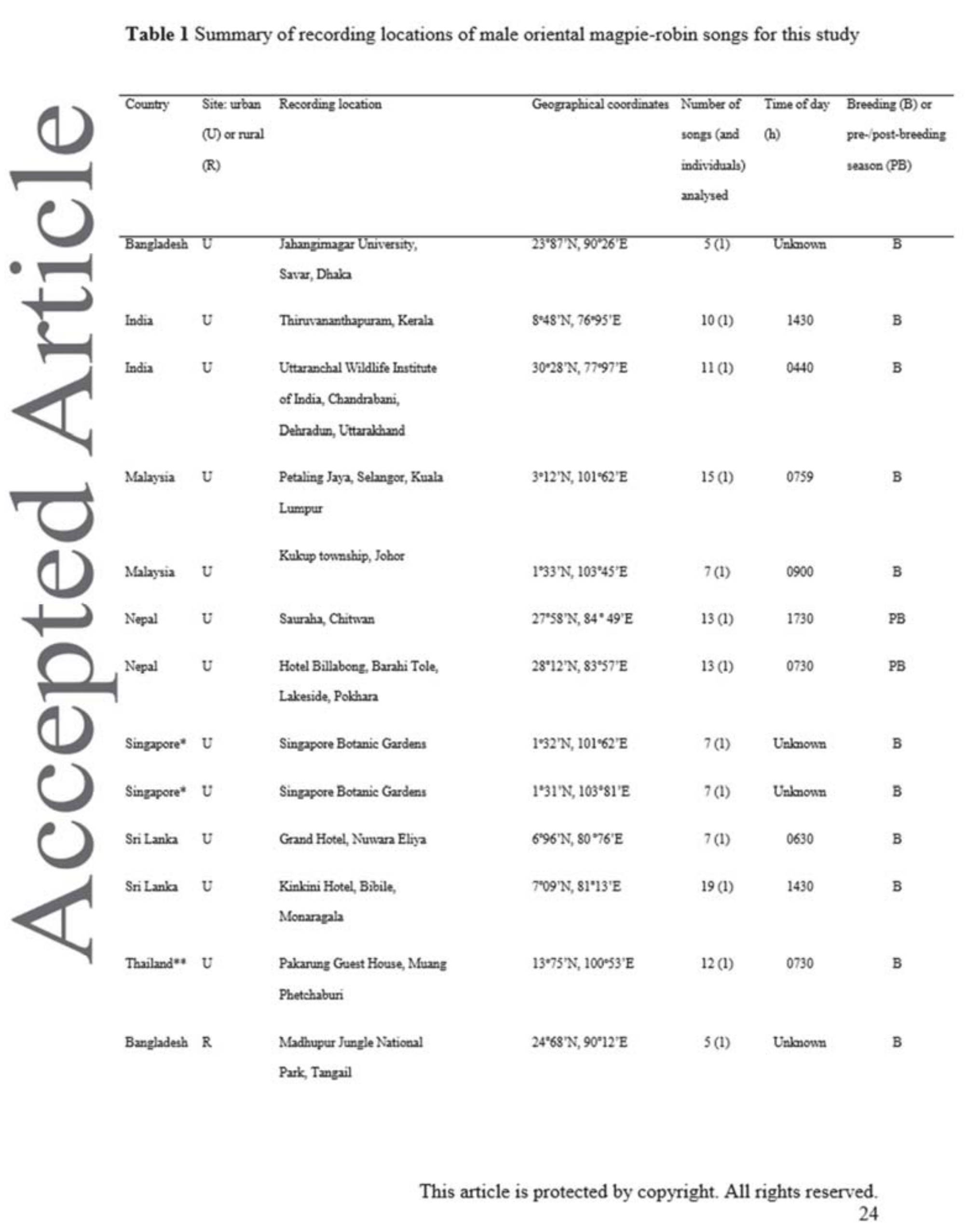

Table 1 Summary of recording locations of male oriental magpie-robin songs for this study

Country	Site: urban (U) or rural (R)	Recording location	Geographical coordinates	Number of songs (and individuals) analysed	Time of day (h)	Breeding (B) or pre-/post-breeding season (PB)
Bangladesh	U	Jahangirnagar University, Savar, Dhaka	23°87'N, 90°26'E	5 (1)	Unknown	B
India	U	Thiruvananthapuram, Kerala	8°48'N, 76°95'E	10 (1)	1430	B
India	U	Uttaranchal Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand	30°28'N, 77°97'E	11 (1)	0440	B
Malaysia	U	Petaling Jaya, Selangor, Kuala Lumpur	3°12'N, 101°62'E	15 (1)	0759	B
Malaysia	U	Kukup township, Johor	1°33'N, 103°45'E	7 (1)	0900	B
Nepal	U	Sauraha, Chitwan	27°58'N, 84° 49'E	13 (1)	1730	PB
Nepal	U	Hotel Billabong, Barahi Tole, Lakeside, Pokhara	28°12'N, 83°57'E	13 (1)	0730	PB
Singapore*	U	Singapore Botanic Gardens	1°32'N, 101°62'E	7 (1)	Unknown	B
Singapore*	U	Singapore Botanic Gardens	1°31'N, 103°81'E	7 (1)	Unknown	B
Sri Lanka	U	Grand Hotel, Nuwara Eliya	6°96'N, 80°76'E	7 (1)	0630	B
Sri Lanka	U	Kinkini Hotel, Bibile, Monaragala	7°09'N, 81°13'E	19 (1)	1430	B
Thailand**	U	Pakarung Guest House, Muang Phetchaburi	13°75'N, 100°53'E	12 (1)	0730	B
Bangladesh	R	Madhupur Jungle National Park, Tangail	24°68'N, 90°12'E	5 (1)	Unknown	B

India	R	Evergreen forest, Kihim, Maharashtra	18°44'N, 72°96'E	18 (1)	Unknown	B
Malaysia	R	Lukut Mangrove Forest, Negeri Sembilan	2°86'N, 102°27'E	9 (1)	0750	B
Malaysia	R	Petaling Jaya, Sarangor	4°72'N, 102°38'E	11 (1)	0710	B
Malaysia	R	Teman Nagara National Park	4°07'N, 102°46'E	10 (1)	0715	B
Nepal	R	Khudi, Lamjung District, Gandaki Zone	14°35'N, 98°92'E	11 (1)	1030	PB
Nepal	R	Koshi Tappu Wildlife Reserve, Terai	26°05'N, 86°83'E	5 (1)	0630	B
Nepal	R	Five miles north of Mugling	27°93'N, 84°58'E	7 (1)	0915	B
Sri Lanka	R	Near Gal Oya National Park, Inginiyagala, Monaragala	7°23'N, 81°31'E	18 (1)	1607	B
Thailand	R	Hellfire Pass, Kanchanaburi	14°29'N, 99°00'E	9 (1)	900	B
Thailand**	R	Hellfire Pass, Kanchanaburi	14°37'N, 98°95'E	9 (1)	1130	B

*As Singapore was an urban site only we subsequently added an extra rural site from neighbouring Malaysia (Lukut Mangrove Forest, Negeri Sembilan, approximately 159 miles (256 km) from Singapore, using the great circle formula)

**Subspecies: *Copsychus saularis erimelas*

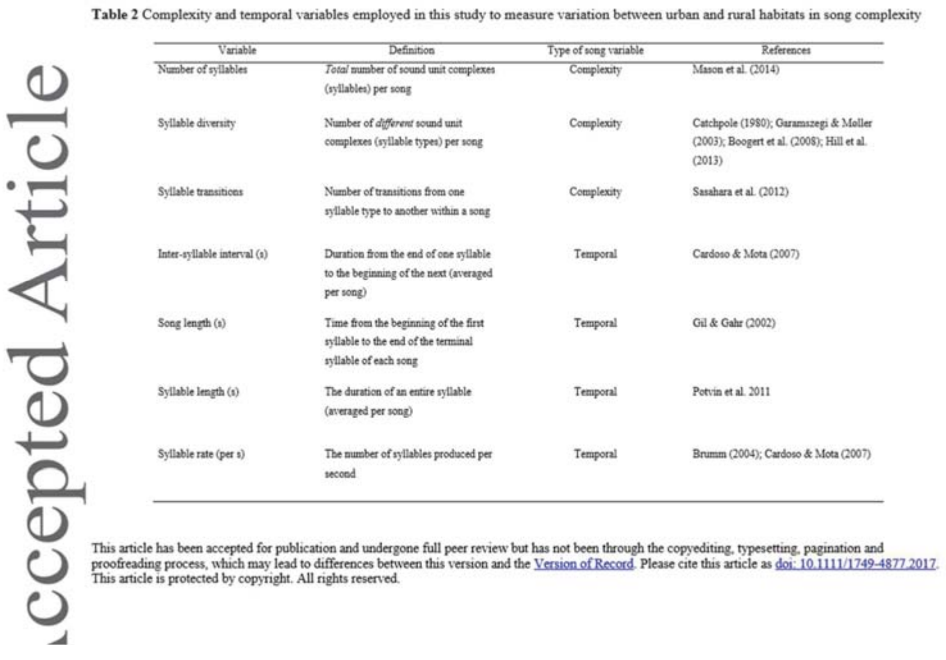

Table 2 Complexity and temporal variables employed in this study to measure variation between urban and rural habitats in song complexity

Variable	Definition	Type of song variable	References
Number of syllables	*Total* number of sound unit complexes (syllables) per song	Complexity	Mason et al. (2014)
Syllable diversity	Number of *different* sound unit complexes (syllable types) per song	Complexity	Catchpole (1980); Garamszegi & Møller (2003); Boogert et al. (2008); Hill et al. (2013)
Syllable transitions	Number of transitions from one syllable type to another within a song	Complexity	Sasahara et al. (2012)
Inter-syllable interval (s)	Duration from the end of one syllable to the beginning of the next (averaged per song)	Temporal	Cardoso & Mota (2007)
Song length (s)	Time from the beginning of the first syllable to the end of the terminal syllable of each song	Temporal	Gil & Gahr (2002)
Syllable length (s)	The duration of an entire syllable (averaged per song)	Temporal	Potvin et al. 2011
Syllable rate (per s)	The number of syllables produced per second	Temporal	Brumm (2004); Cardoso & Mota (2007)

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Table 3 The descriptive statistics of the 3 song complexity and 4 temporal variables used to measure song differences between urban and rural populations of oriental magpie-robin. The mean values for each variable are averages of the sum of all individuals within their respective category (urban or rural)

Variable	Mean ± SD	
	Urban	Rural
Number of syllables per song	8.08 ± 2.37	7.9 ± 3.02
Syllable diversity per song	7.32 ± 1.93	6.35 ± 2.47
Syllable transitions per song	6.54 ± 2.09	5.8 ± 2.39
Inter-syllable interval* (s)	0.12 ± 0.02	0.09 ± 0.04
Song length** (s)	2.3 ± 0.58	1.72 ± 0.46
Syllable length (s)	0.21 ± 0.17	0.16 ± 0.14
Syllable rate (per s)	3.58 ± 0.26	4.55 ± 0.34

*Univariate Student's t-test: $t = -2.75$; $P = 0.012$

**Univariate Mann-Whitney-Wilcoxon test: $W = 98$; $P = 0.037$

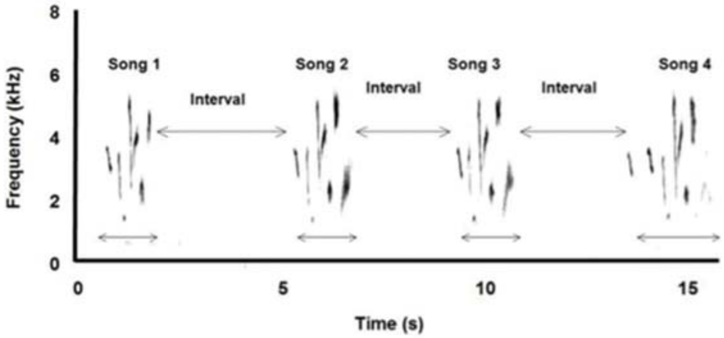

Figure 1 An example of the general pattern of oriental magpie-robin vocalizations. Each song (a consecutive series of syllables) is followed by an interval of silence before the next song begins.

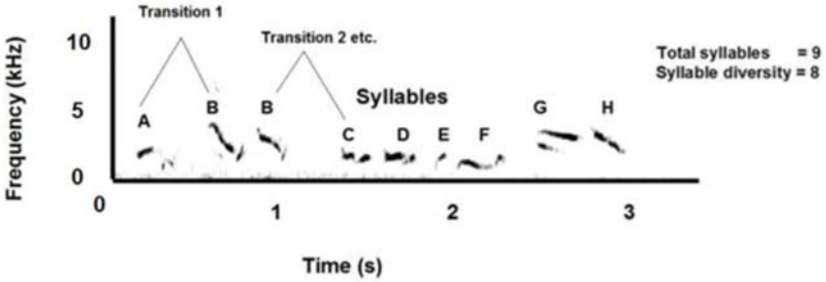

Figure 2 An oriental magpie-robin song spectrogram showing how the number of syllables, syllable diversity, and the number of syllable transitions of each song was calculated. Song length was calculated by the time axis. The (mean) inter-syllable interval length of each song was calculated by dividing the total duration of intervals in the song by the total number of intervals in the song. Similarly, the mean syllable length of each song was calculated by dividing the total duration of syllables by the total number of syllables in the song.

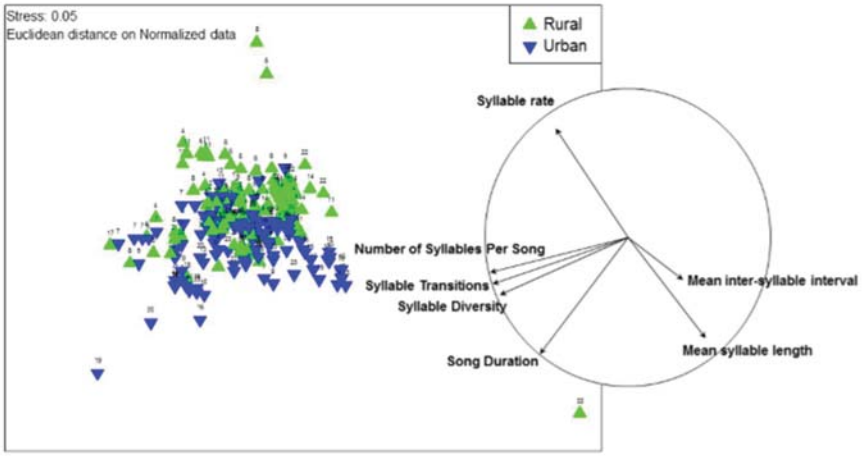

Figure 3 A NMDS biplot visualizing the relative differences of urban and rural songs (all 238 songs, labeled per bird) using all 7 variables employed in this study. The inset unit circle shows the Pearson rank correlations of song variables with each of the axes.

Seasonal variation in the song structure of tui (*Prosthemadera novaeseelandiae*)

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Abstract: Birdsong has evolved to help individual birds attract mates and defend territories. The breeding season is a critical period in the life history of many songbird species and previous studies indicate that bird vocal behaviour changes at the onset of breeding season. In this study, we compared the complex songs of a seasonal-breeding New Zealand honeyeater, the tui (*Prosthemadera novaeseelandiae*), between breeding and non-breeding seasons. We found that males' songs in the breeding season contained significantly greater proportions of trill components compared with songs in the non-breeding season. Trill rate and consistency may be related to individual quality and therefore might signal to rivals and potential mates the quality of the singer. This is the first study to show the differences in singing behaviour of tui between seasons and provides a basis for future studies to explore the drivers of this seasonal variation in song behaviour.

Keywords: birdsong, breeding season, non-breeding season, trills

Introduction

Song plays a vital role in communication in songbirds and is primarily for territorial defence and mate attraction (Catchpole & Slater 2008). Some species of birds, particularly in the tropics, may have year-round breeding, and may also sing throughout the year (Kunkel 1974; Stutchbury & Morton 2008). Other species, however, have more well-defined breeding seasons and so their song outputs may be confined to these times (Langmore 1998) – a critical period in the life history of many species (Johnston & Odum 1956). Alternatively, some species also sing throughout the year despite not breeding, but change their song properties between reproductive and non-reproductive periods. For example, the songs of wild male island canaries (*Serinus canaria*) were longer and had more syllable repetition in summer than in the non-breeding season (Leitner et al. 2001). Specifically, a decrease in whistle-type syllables and increases in rapid, frequency-modulated syllables was detected (known as trill syllables) (Leitner et al. 2001). Evidence suggests that male songs with more rapid rates of trill production are more sexually attractive to female canaries than songs with low trill rates and induce more female copulatory displays (reviewed in Podos et al. 2004). Trills are likely to be costly to produce and therefore may provide honest signals of physical and genetic quality of the singer (Hennin et al. 2009). Moreover, trill consistency or stereotypy is a vocal characteristic that has been linked with extra-pair success in male house wrens (*Troglodytes aedon*) (Cramer 2013). Therefore, trill production and consistency are likely to be sexually selected, and so should be produced at higher rates by individuals during the breeding season.

In many species, song complexity (measured by the number of different syllables produced; Boogert et al. 2008) is related to male attractiveness to females (Badyaev et al. 2002). Moreover, song complexity may reflect parental ability, reproductive success, and overall male quality (Buchanan & Catchpole 2000; Woodgate et al. 2011, 2012; Soma & Garamszegi 2011). For example, female common grackles (*Quiscalus quiscula*) prefer to breed with males that have large song repertoires (Searcy 1992). In pied flycatchers (*Ficedula hypoleuca*) early arriving-males also sang more complex songs than later-arriving males (Alatalo et al. 1986; Lampe & Espmark 1994); suggesting that song complexity may be an indicator of male quality.

In some species, birds also produce songs in the non-breeding season. However, songs during this time may be different from those sung during the breeding season (e.g. quieter, in song sparrows *Melospiza melodia*; Maddison et al. 2012). The difference in songs between breeding and non-breeding season may be due to hormonally-mediated changes in anatomy (Riters et al. 2000). For example, seasonal variation in song can be attributed to the plasticity of song control nuclei, such as the high vocal centre (HVC), in songbird brains (Catchpole & Slater 2008). The volume of the HVC has been observed to change seasonally, and increased testosterone levels during the breeding season concomitantly increase the volume of the song nuclei (Ball et al. 2004). This in turn impacts the muscles of the syrinx (e.g. DeVoogd 1991) and subsequently birds' vocal behaviour. These seasonal changes in songbird neuroendocrinology play a key role in the seasonal differences of their song production (Ball et al. 2004).

Other song parameters apart from trills may also be subject to sexual selection and therefore may vary between seasons. For example, maximum frequency (F_{max}) and frequency of maximum amplitude (FMA) have both been shown to be correlated with reproductive success and genetic quality in rock sparrows (*Petronia petronia*) (Nemeth et al. 2012) and zebra finches (*Taeniopygia guttata*) (Woodgate et al. 2012). Therefore, male songbirds may display seasonal variation in these traits in order to stimulate females to breed, and to maximise their reproductive success. Although seasonal changes in bird songs have been found in many species, few studies have quantitatively examined seasonal variation in song structure.

Bergquist (1989) found that winter songs of male tūī (*Prosthemadera novaeseelandiae*), a species that has highly complex vocalisations, generally consisted of a series of phrases that were repeated. During the breeding season male

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songs were comprised of sequences of many loud phrases sung repeatedly, but with pauses, sporadic clicks, and whistles between phrases. Although these differences are noticeable to the human ear, there has been no detailed quantitative analysis of the seasonal variation of tūī songs. In this study, we examined the characteristics of tūī songs at the population level during breeding season and compared them to those in the non-breeding season. We predicted there would be a significant difference in song structure between seasons. Specifically, we predicted that tūī would produce a greater proportion of trills, a higher F_{max}, syllable diversity, and higher FMA in the breeding season than in the non-breeding season (after Woodgate et al. 2012, see above). Trills are costly to produce and may, therefore, be used to signal male quality during the breeding season.

Methods

Study species and site

Tūī are honeyeaters endemic to New Zealand. They are common throughout the three main islands, but are scarce on the east coast of the South Island (Gill et al. 2010). Tūī are also found in the subantarctic Auckland Islands and the subtropical Kermadec Islands (Gill et al. 2010). Breeding begins in September and finishes in February. Upon pairing the female starts building a nest (Heather & Robertson 2005). There are also extremely high levels of extra-pair paternity in this species (S. J. Wells, unpubl. data).

Male songs change noticeably at the start of the breeding season. From high vantage points tūī produce long-range-broadcast songs, which may function for territorial defence and mate attraction. Long-range tūī songs are complex and there is significant geographical variation among populations (Hill et al. 2013), and between sexes (Bergquist 1989). Tūī also produce short-range songs that often have aggressive contexts (Hill 2011), but long-range-song bouts in tūī are significantly longer than these short-range bouts (SDH unpubl. data).

Our study was conducted at Tāwharanui Regional Park (36°22′ S, 174°50′ E), a 588-ha predator-free sanctuary situated on the Tāwharanui Peninsula, 90 km north of central Auckland, New Zealand. Tāwharanui Regional Park consists of predominantly grazed pasture with patches of regenerating mānuka (*Leptospermum scoparium*) bush and some areas of mature coastal forest remnants (ARC 2009). The canopy tree species in these forest remnants include pōhutukawa (*Metrosideros excelsa*), kauri (*Agathis australis*) and puriri (*Vitex lucens*) (ARC 2009). Recordings were made at four locations within the park: Jones Bay, Anchor Bay, Ecology Bush, and Māori Bay (Fig. 1).

Recording procedure

We recorded tūī broadcast songs during the breeding season (October 2010 – January 2011) and non-breeding season (July–August 2010; August 2012 and May 2013). Songs were recorded between 0800 and 1500 hours in both seasons to standardise sampling times and sampling effort. We sampled tūī songs by walking slowly along marked tracks in one direction to locate tūī. We started a recording session when we located a singing male and ended it when the tūī either ceased singing or flew away. We stood between 4 and 10 m from a bird when we recorded it. For each recording, we noted the time, the bird's location, band combination (if it was banded), behaviour, and song type.

In order to minimise the chances of recording the same individual more than once in the same season, we recorded only one bird per location. Male tūī are highly territorial during the breeding season and sing largely within their own territories during this time. Therefore, recording birds in different locations was unlikely to result in recording the same bird twice. The minimum distance between recording locations was 300 m.

We recorded tūī songs using a Sony MZ-NHF800 Portable High-Definition MiniDisc Recorder (Sony, Park Ridge, NJ) or a Marantz PMD620 solid-state digital recorder (Marantz, Kanagawa, Japan), which were both paired with a Sennheiser ME67 shotgun long-range directional microphone (Sennheiser, Old Lyme, CT). All songs were recorded at a sampling frequency of 44.1 kHz at a resolution of 16 bits. The frequency response range of the long-range microphone was 40 Hz to 20 kHz. MiniDisc sound files were converted to wave (.wav) files using the software 'Sonic Stage' (Sony Corporation, Tokyo, Japan).

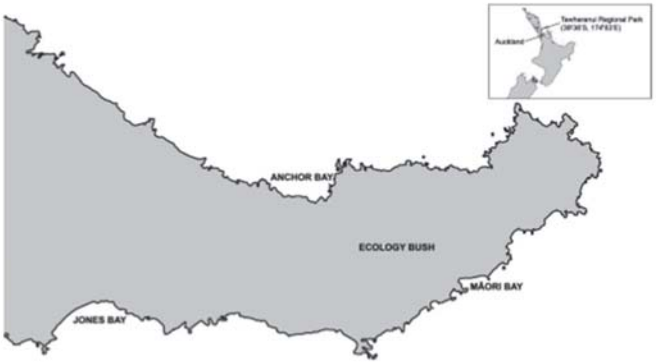

Figure 1. Map of Tāwharanui Regional Park with the four recording locations of Anchor Bay, Jones Bay, Ecology Bush, and Māori Bay, and (inset) a map of New Zealand's North Island showing the location of Tāwharanui in relation to Auckland City.

Comparison of spectral characteristics between seasons

One representative long-range song with a clean, high quality recording was analysed per individual. The song spectrograms were digitised and parameters measured using Raven Pro 1.4 Beta Version software (Cornell Lab of Ornithology, Ithaca, NY, USA). For all recordings, spectrograms were created by Discrete Fourier Transform (DFT) with a Hann window. The frame length was set at 256 points. Additionally, a 50% frame overlap with hop size of 2.9 milliseconds was used. Frequency grid spacing of 172 Hz was also employed and the bandwidth was set at 3 dB. To investigate the variation of song spectral characteristics of male tūī between seasons, we used Raven Pro to extract 10 variables for analysis (Table 1; Fig. 2). These variables have been employed for characterising variation in bird songs in previous studies (Mennill & Rogers 2006; Hennin et al. 2009).

Analysis

For each season, we tested whether there was a difference between morning and afternoon song, using a one-way permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001). PERMANOVA is a non-parametric distance-based analysis of variance that uses permutation procedures to test hypotheses. In this case, the Euclidean distance measure was used. In order to remove skewness, we used a ln(x) + 0.01 transformation for five variables: number of notes, song duration, trill duration, FMA, and syllable diversity. The 10 variables from the breeding and non-breeding seasons were examined using one-way PERMANOVA to detect whether song structure between seasons was significantly dissimilar. PERMANOVA was also used to detect any significant differences between morning song (0800–1200 hours) and afternoon song (1200–1500

Table 1. Definition of the 10 variables employed to compare songs of tūī between seasons.

Song variable	Definition
Number of notes	Number of individual notes (fundamental sound units) per song. A note alone without a repeat is considered a syllable. Multiple notes repeated consecutively are considered one syllable
Song duration (seconds)	Time from the beginning of the first note to the end of the terminal note of each chosen song
Trill duration (seconds)	Cumulative total length of repeated, high-frequency-modulation portions per song
Trill percentage	Percentage of each song that contains rapid, repeated, high-frequency-modulation notes. Computed by dividing song duration with the amount of time (seconds) trills are produced within a song
Frequency of maximum amplitude (FMA) (kHz)	Frequency of the time point within the song that contains the highest level of energy
Syllable diversity	Number of *different* sound unit complexes (syllables) per song. Syllables can be comprised of several notes
Maximum frequency (kHz)	The highest pitch produced in a song
Minimum frequency (kHz)	The lowest pitch produced in a song
Mean frequency (kHz)	Overall average pitch of sounds per song
Bandwidth (kHz)	The difference between maximum and minimum frequency

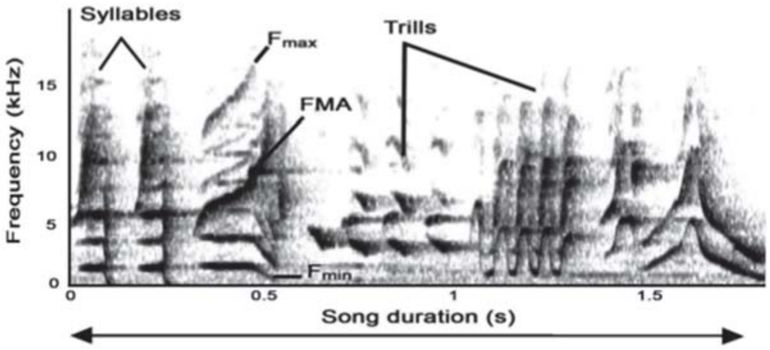

Figure 2. A spectrogram of the long-range song of a mainland male tūī (*Prosthemadera novaeseelandiae*), showing the variables employed in this study.

hours) and within the same season (breeding season: September–February; non-breeding season: March–August). This allowed us to assess whether all data from the same season could be pooled for further analysis. The conservative Bonferroni correction for multiple testing was used for all multivariate analyses. PERMANOVA was performed using PAST software (version 2.17b) (Hammer et al. 2001).

Four variables – trill percentage, F_{max}, syllable diversity and FMA – were compared between seasons. These four variables were chosen as these song characteristics have been linked with reproductive success in other songbird species. We used non-parametric Mann–Whitney-Wilcoxon tests, as the data were non-normally distributed, to test our hypotheses that tūī would produce a (1) greater proportion of trills, (2) higher F_{max}, (3) higher syllable diversity, and (4) higher FMA in the breeding season than in the non-breeding season. These tests were conducted using SPSS (version 20.0; SPSS, Chicago, IL, USA). All statistical tests were two-tailed and P values < 0.05 were interpreted as significant.

Results

In total, 27 birds were recorded (14 in the breeding season and 13 in the non-breeding season). Five of the birds we recorded during the breeding season were banded, while only a single banded bird was recorded in the non-breeding season.

Within-season variation in tūī song structure

Results from a one-way PERMANOVA test (9999 permutations) showed no significant difference in song structure between songs recorded in the morning and those in the afternoon for both the breeding season ($n = 6, 8$; Pseudo $F_{3,14} = 0.5314$, $P = 0.743$) and non-breeding season ($n = 9, 4$; Pseudo $F_{2,13} = 0.589$, $P = 0.612$). Therefore the data obtained from the mornings and afternoons were pooled for further analysis.

The PERMANOVA test (9999 permutations) showed no significant difference in song structure within seasons for either the breeding season ($n = 14$; Pseudo $F_{3,14} = 0.473$, $P = 0.982$) or non-breeding season ($n = 13$; Pseudo $F_{2,13} = 1.42$, $P = 0.257$). Therefore the data within a season were pooled for further analysis.

Between-season variation in tūī song structure

A one-way PERMANOVA test revealed a significant difference in the ranked distance in tūī song structure (using all ten variables) between the breeding season and the non-breeding season ($n = 14, 13$, Pseudo $F_{16,27} = 2.856$, $P = 0.044$, 9999 permutations). This suggested that tūī alter their song structure at the onset of the breeding season.

The results of the Mann–Whitney-Wilcoxon tests showed that there was a significantly greater proportion of trills in breeding season songs compared to non-breeding season songs ($W = 98$, $P = 0.001$) (Table 2; Fig. 3). However, there were no significant differences between seasons in F_{max}, syllable diversity, and FMA (Table 2).

Table 2. Descriptive statistics of four tūī song variables between seasons at Tāwharanui.

Variable	Median; Interquartile range		Range		Statistical values from Mann–Whitney –Wilcoxon tests	
	Breeding season	Non-breeding season	Breeding season	Non-breeding season	W	P
Proportion of song containing trills (%)	56; 39–75	24; 7–38	18, 93	0, 61	98	0.001
FMA (kHz)	1.60; 1.47–1.72	1.54; 1.31–1.55	1.37, 1.86	1.03, 1.72	98	0.069
Syllable diversity	18; 16–26	15.5; 6–28	14, 56	2, 31	156.5	0.220
F_{max} (kHz)	15.11; 12.41–18.90	15.36; 11.16–18.46	11.50, 19.96	4.95, 19.84	193.5	0.905

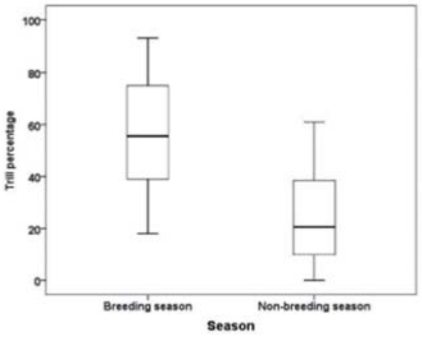

Figure 3. Trill percentage ($P = 0.001$) in songs of male tūī (*Prosthemadera novaeseelandiae*) in the breeding season compared with the non-breeding season at Tāwharanui Regional Park. On average, the trills were sung at twice the rate in breeding-season songs than in non-breeding-season songs.

Discussion

We found a significantly greater proportion of trills in male tūī songs in the breeding season than the non-breeding season, which is consistent with our hypothesis, but no difference in FMA, syllable diversity or F_{max} between seasons (Table 2). That males produced a higher proportion of trills in the breeding season suggests this song characteristic may be sexually selected. Trills are complex, rapidly repeated syllables that are costly to produce (Suthers & Goller 1998), and are considered honest signals of male phenotypic quality and may advertise the physical and genetic condition of callers (Ballentine et al. 2004; Ballentine 2009; Sewall et al. 2010). Males that produce a higher number of trills during the breeding season may therefore be demonstrating their phenotypic quality and suitability as mates to females. For example, swamp sparrows (*Melospiza georgiana*) that included a higher proportion of trills within their songs have been shown to have greater breeding success than other males (Ballentine et al. 2004). Furthermore, in island canaries, males altered their songs during the breeding season so that they contained more trills (Leitner et al. 2001). Therefore, it is possible that males in our study population were using trills to advertise their quality to potential mates.

Trills may also signal aggressive intent in birds and may elicit more aggressive responses from rival males. For example, male nightingales (*Luscinia megarhynchos*) that produced trills with broad bandwidths elicited greater aggressive responses from rival males than those who produced trills with narrower bandwidths (Schmidt et al. 2008). In males that became paired during the breeding season, there was also a positive correlation between increased trill production (from a simulated model) and increased aggression (Schmidt et al. 2008). Therefore, we encourage further research to assess the significance of trills in reproductive and territorial contexts in New Zealand passerines (including tūī).

Although male tūī produced more trills in the breeding season, there were no differences in the other frequency parameters (FMA andF_{max}) recorded between seasons. This lack of difference in frequency parameters might indicate the tūī in our study were subject to physical or energetic constraints. For example, silvereyes (*Zosterops lateralis*) increased the F_{max} and F_{min} during the dawn chorus on days when they had access to supplementary food (Barnett & Briskie 2007). This suggests that the quality of bird songs may be related to the birds' energy levels. However, the relationships between birds' energy levels and specific song parameters within species require further research.

The seasonal difference in birds' song was likely to be mediated hormonally. For example, blood titres of testosterone have been directly correlated with levels of competition in birds and are increased during the breeding season, for example in canaries (Bolhius & Gahr 2006). Furthermore, studies in zebra finch and male grey partridge (*Perdix perdix*) show that testosterone can affect specific features of bird vocalisations, which may enable females to assess male quality (Fusani et al. 1994). Future studies could experimentally investigate the individual differences in birds' endocrinological profiles and the quality of their song displays and relate this to individual reproductive success.

In conclusion, we found that male tūī songs contained a greater proportion of trills in the breeding season compared with their songs in the non-breeding season – the first study to have shown how tūī songs change between seasons. These seasonal vocal changes may be related to male resource-holding potential and mate choice. However, we encourage further studies to investigate the adaptive significance of seasonal variations in tūī songs.

Acknowledgements

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References

Alatalo RV, Lundberg A, Glynn C 1986. Female pied flycatchers choose territory quality and not male characteristics. Nature 323: 152–153.

Anderson MJ 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

ARC 2009. Draft terrestrial biodiversity monitoring plan October 2009. Auckland, Auckland Regional Council. 59 p.

Badyaev AV, Hill GE, Weckwort BV 2002. Species divergence in sexually selected traits: increase in song elaboration is related to decrease in plumage ornamentation in finches. Evolution 56: 412–419.

Ball GF, Auger CJ, Bernard DJ, Charlier TD, Sartor JJ, Riters LV, Balthazart J 2004. Seasonal plasticity in the song control system. Multiple brain sites of steroid hormone action and the importance of variation in song behavior. Annals of the New York Academy of Sciences 1016: 586–610.

Ballentine B 2009. The ability to perform physically challenging songs predicts age and size in male swamp sparrows (*Melospiza georgiana*). Animal Behaviour 77: 973–978.

Ballentine B, Hyman J, Nowicki S 2004. Vocal performance influences female response to male bird song: an experimental test. Behavioral Ecology 15: 163–168.

Barnett CA, Briskie JV 2007. Energetic state and the performance of dawn chorus in silvereyes (*Zosterops lateralis*). Behavioral Ecology and Sociobiology 61: 579–587.

Bergquist CAL 1989. Tui siociodynamics: foraging behaviour, social organisation, and use of song by tui in an urban area. Unpublished PhD thesis, University of Auckland, New Zealand. 79 p.

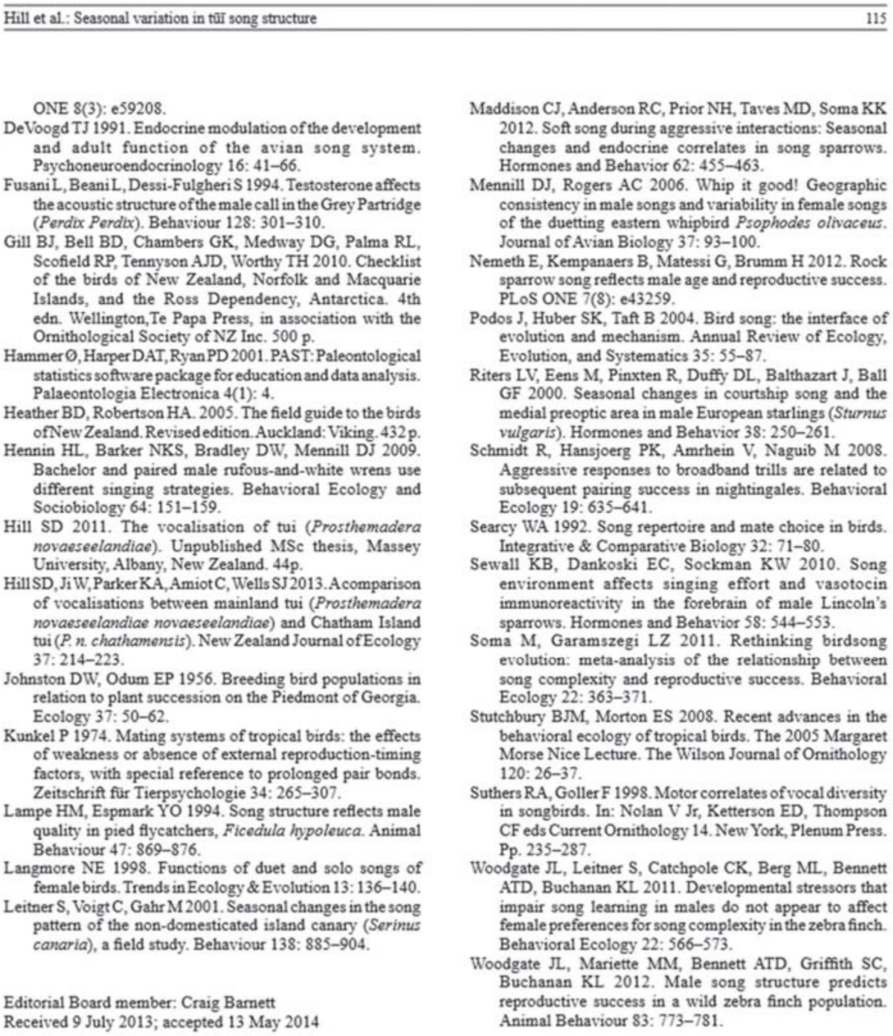
Bolhius JJ, Gahr M 2006. Neural mechanisms of birdsong memory. Nature Reviews Neuroscience 7: 347–357.

Boogert NJ, Giraldeau L-A, Lefebvre L 2008. Song complexity correlates with learning ability in zebra finch males. Animal Behaviour 76: 1735–1741.

Buchanan KL, Catchpole CK 2000. Song as an indicator of male parental effort in the sedge warbler. Proceedings of the Royal Society B – Biological Sciences 267: 321–326.

Catchpole CK, Slater PJB 2008. Bird song: biological themes and variations. 2nd edn. Cambridge University Press. 335 p.

Cramer ERA 2013. Physically challenging song traits, male quality, and reproductive success in house wrens. PLoS



ONE 8(3): e59208.

DeVoogd TJ 1991. Endocrine modulation of the development and adult function of the avian song system. Psychoneuroendocrinology 16: 41–66.

Fusani L, Beani L, Dessi-Fulgheri S 1994. Testosterone affects the acoustic structure of the male call in the Grey Partridge (*Perdix Perdix*). Behaviour 128: 301–310.

Gill BJ, Bell BD, Chambers GK, Medway DG, Palma RL, Scofield RP, Tennyson AJD, Worthy TH 2010. Checklist of the birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica. 4th edn. Wellington,Te Papa Press, in association with the Ornithological Society of NZ Inc. 500 p.

Hammer Ø, Harper DAT, Ryan PD 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 4.

Heather BD, Robertson HA. 2005. The field guide to the birds of New Zealand. Revised edition. Auckland: Viking. 432 p.

Hennin HL, Barker NKS, Bradley DW, Mennill DJ 2009. Bachelor and paired male rufous-and-white wrens use different singing strategies. Behavioral Ecology and Sociobiology 64: 151–159.

Hill SD 2011. The vocalisation of tui (*Prosthemadera novaeseelandiae*). Unpublished MSc thesis, Massey University, Albany, New Zealand. 44p.

Hill SD, Ji W, Parker KA, Amiot C, Wells SJ 2013. A comparison of vocalisations between mainland tui (*Prosthemadera novaeseelandiae novaeseelandiae*) and Chatham Island tui (*P. n. chathamensis*). New Zealand Journal of Ecology 37: 214–223.

Johnston DW, Odum EP 1956. Breeding bird populations in relation to plant succession on the Piedmont of Georgia. Ecology 37: 50–62.

Kunkel P 1974. Mating systems of tropical birds: the effects of weakness or absence of external reproduction-timing factors, with special reference to prolonged pair bonds. Zeitschrift für Tierpsychologie 34: 265–307.

Lampe HM, Espmark YO 1994. Song structure reflects male quality in pied flycatchers, *Ficedula hypoleuca*. Animal Behaviour 47: 869–876.

Langmore NE 1998. Functions of duet and solo songs of female birds. Trends in Ecology & Evolution 13: 136–140.

Leitner S, Voigt C, Gahr M 2001. Seasonal changes in the song pattern of the non-domesticated island canary (*Serinus canaria*), a field study. Behaviour 138: 885–904.

Editorial Board member: Craig Barnett
Received 9 July 2013; accepted 13 May 2014

Maddison CJ, Anderson RC, Prior NH, Taves MD, Soma KK 2012. Soft song during aggressive interactions: Seasonal changes and endocrine correlates in song sparrows. Hormones and Behavior 62: 455–463.

Mennill DJ, Rogers AC 2006. Whip it good! Geographic consistency in male songs and variability in female songs of the duetting eastern whipbird *Psophodes olivaceus*. Journal of Avian Biology 37: 93–100.

Nemeth E, Kempanaers B, Matessi G, Brumm H 2012. Rock sparrow song reflects male age and reproductive success. PLoS ONE 7(8): e43259.

Podos J, Huber SK, Taft B 2004. Bird song: the interface of evolution and mechanism. Annual Review of Ecology, Evolution, and Systematics 35: 55–87.

Riters LV, Eens M, Pinxten R, Duffy DL, Balthazart J, Ball GF 2000. Seasonal changes in courtship song and the medial preoptic area in male European starlings (*Sturnus vulgaris*). Hormones and Behavior 38: 250–261.

Schmidt R, Hansjoerg PK, Amrhein V, Naguib M 2008. Aggressive responses to broadband trills are related to subsequent pairing success in nightingales. Behavioral Ecology 19: 635–641.

Searcy WA 1992. Song repertoire and mate choice in birds. Integrative & Comparative Biology 32: 71–80.

Sewall KB, Dankoski EC, Sockman KW 2010. Song environment affects singing effort and vasotocin immunoreactivity in the forebrain of male Lincoln's sparrows. Hormones and Behavior 58: 544–553.

Soma M, Garamszegi LZ 2011. Rethinking birdsong evolution: meta-analysis of the relationship between song complexity and reproductive success. Behavioral Ecology 22: 363–371.

Stutchbury BJM, Morton ES 2008. Recent advances in the behavioral ecology of tropical birds. The 2005 Margaret Morse Nice Lecture. The Wilson Journal of Ornithology 120: 26–37.

Suthers RA, Goller F 1998. Motor correlates of vocal diversity in songbirds. In: Nolan V Jr, Ketterson ED, Thompson CF eds Current Ornithology 14. New York, Plenum Press. Pp. 235–287.

Woodgate JL, Leitner S, Catchpole CK, Berg ML, Bennett ATD, Buchanan KL 2011. Developmental stressors that impair song learning in males do not appear to affect female preferences for song complexity in the zebra finch. Behavioral Ecology 22: 566–573.

Woodgate JL, Mariette MM, Bennett ATD, Griffith SC, Buchanan KL 2012. Male song structure predicts reproductive success in a wild zebra finch population. Animal Behaviour 83: 773–781.

A comparison of vocalisations between mainland tui (*Prosthemadera novaeseelandiae novaeseelandiae*) and Chatham Island tui (*P. n. chathamensis*)

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Abstract: Vocalisations are important for territorial defence, mate attraction, and species recognition in many songbirds. Comparative studies on the songs of birds between islands and mainland populations provide insight into the evolution of vocal communication in terms of both ecological and social factors. We compared the vocalisations of tui (*Prosthemadera novaeseelandiae novaeseelandiae*), an endemic honeyeater from New Zealand's mainland, with those of a subspecies from the remote Chatham Islands (*P. n. chathamensis*). Song spectral variables of male long-range 'broadcasting' songs differed between mainland and Chatham Island tui populations. Songs recorded from five mainland populations had significantly greater syllable diversity and produced a higher percentage of trills than those from the Chatham Islands. These characteristics have been revealed in past studies as being sexually selected traits positively correlated with male genetic diversity. The acoustic adaptation hypothesis could also help to elucidate the presence of a significantly higher percentage of trill components in the songs of the mainland population, as the mainland study sites in general contained more areas of open vegetation, where trills transmit more effectively than in dense forest, which dominated the sampling sites on the Chatham Islands. Future research into the variation of tui song complexity in relation to population size, geographic isolation, and habitat structure between different mainland sites would provide further insight into links between selective pressures and vocal complexity.

Keywords: diversity; island; song; syllable; trill; variation

Introduction

Geographical variation in bird song is common in many species (Marler & Tamura 1962; Baker & Cunningham 1985). These differences can be on a microgeographic (Payne 1978; Leader et al. 2000) or macrogeographic scale (Benedict & Bowie 2009; Pitocchelli 2011). Geographical differences in bird vocalisations consist of variations in syllable (sound unit) and song structure (the patterns of song delivery), similar to regional dialects or accents in humans (Kroodsma 2005). In previous research, geographical variation of vocalisations has been observed in a wide number of species such as song sparrow (*Melospiza melodia*) (Peters et al. 2000), white-crowned sparrow (*Zonotrichia leucophrys*) (Derryberry 2011), blue tit (*Parus caeruleus*) (Doutrelant et al. 1999), and Eurasian nuthatch (*Sitta europaea*) (White 2012). New Zealand avian species have also been shown to exhibit regional variation in their vocalisations (bellbird *Anthornis melanura*, Brunton et al. 2008; hihi or stitchbird *Notiomystis cyncta*, Ranjard 2010; North Island saddleback *Philesturnus rufusater*, Parker et al. 2012; kōkako *Callaeas cinerea*, Brown et al. 2004, Rowe & Bell 2007; and kea *Nestor notabilis*, Bond & Diamond 2005).

Several hypotheses have been proposed to explain the evolution of regional song variation. These include the 'local adaptation hypothesis', which suggests females gain fitness advantages by breeding with birds from their natal regions rather than with birds from regions further away (Podos & Warren 2007). Dialects may function to induce birds to breed close to their natal areas as females are more attracted to males that sing local dialect, thereby promoting assortative mating (Baptista & Morton 1982).

The 'acoustic adaptation hypothesis' proposes that birds inhabiting areas with similar vegetation type will share common song features (Date & Lemon 1993). This hypothesis suggests that the evolution of the acoustic properties of bird songs has been shaped by selection that is dependent upon habitat structure (Boncoraglio & Saino 2007). It predicts that songs that have on average lower frequency, narrower bandwidths, low-frequency whistles, long syllables, and long inter-syllable intervals are more likely to be produced in closed habitats such as rainforests that contain dense and complex vegetation (Nemeth et al. 2006). A more frequent production of high-frequencies, broader bandwidths, high-frequency modulations (trills), short syllables, and short inter-syllable intervals are expected to be produced in areas that are more open (Nemeth et al. 2006), in order to maximise the effective transmission of sound.

The 'social adaptation hypothesis' predicts that males gain fitness advantages by learning songs from dominant local males to maximise their chances of mating and breeding success (Payne et al. 2000). For example, young males of the highly promiscuous village indigobird (*Vidua chalybeata*) will mimic the songs of the older, dominant and reproductively successful male neighbours in order to attract female birds (Payne et al. 2000).

Vocal properties may vary within different habitat types across regions, which in turn may coincide with changes in physiological and morphological features that underpin bird

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song production, such as beak size and syrinx mass (Podos & Warren 2007). This phenotypic plasticity ultimately can contribute to the divergence of species (Slabbekoorn & Smith 2002).

Variation in avian vocalisations has also been previously observed between mainland and isolated island populations of singing honeyeaters (*Lichenostomus virescens*) (Baker et al. 2002), hwamei (*Garrulax canorus*) (Tu & Severinghaus 2004) and bush warblers (*Cettia diphone*) (Hamao & Ueda 2000). Island populations tend to have structurally simpler and less complex songs than their mainland congeners due to factors such as weaker selection pressures towards complex song for the acquisition of breeding partners and/or high quality territories (Hamao & Ueda 2000). Lowered song complexity in island subspecies might also be caused by founder effects, leading to reduced song diversity, as a result of a new population being established by a small number of founders from a larger population (Baker & Moeed 1987; Baker 1996; Parker et al. 2012). Indeed, evidence for a founder effect in song (cultural founder effect) has been observed in the Chatham Island population of chaffinch (*Fringilla coelebs*), an introduced European passerine (Baker & Jenkins 1987).

Song complexity is a sexually selected trait of fundamental importance to successful territory defence and mate attraction in songbirds (Briefer et al. 2010). Syllable repertoire size (syllabic diversity), the length of song, or the production of complex syllables can all be used to measure song complexity in birds (Boogert et al. 2008). Evidence suggests that this complexity is increased within mainland populations due to greater levels of male competition for resources, food, and mates than their island counterparts (Craig & Jenkins 1982). Complexity of vocalisations has also been positively correlated with parental ability (Buchanan & Catchpole 2000), reproductive success (Woodgate et al. 2012) and overall male quality (Buchanan & Catchpole 2000; Soma & Garamszegi 2010).

We examined geographical variation of the highly complex vocalisations of mainland tūī (*Prosthemadera novaeseelandiae novaeseelandiae*) and an isolated subspecies, the Chatham Island tūī (*P. n. chathamensis*). The tūī is a honeyeater species in the Order Passeriformes, Suborder Oscine, and the Family Meliphagidae. There are only two members of Meliphagidae endemic to New Zealand, the bellbird and the tūī (Driskell et al. 2007), the tūī being the larger and more dominant of the two species (Stewart & Craig 1985; Bergquist 1989).

Although common throughout the North Island, South Island, and Stewart Island of New Zealand, tūī are scarce on the east coast of the South Island (Gill et al. 2010). Tūī are also found in the subtropical Kermadec Islands and subantarctic Auckland Islands (Gill et al. 2010). The endangered Chatham Island subspecies (Miskelly et al. 2008) has been subject to recent translocation efforts that have resulted in the reestablishment of Chatham Island tūī onto the main Chatham Island (Bell et al. 2013). Very few recent studies have focused on tūī behavioural ecology, including their vocalisations. One previous study, however, suggested that tūī song exhibits sexual, regional, behavioural, and individual variation (Bergquist 1989).

The Chatham Islands are a volcanic archipelago formed just 3–4 million years ago (Stilwell & Consoli 2012), situated 800 km east of Christchurch, New Zealand. The islands are subject to high levels of endemism with 16 bird species being endemic to the Chatham Islands (Aikman et al. 2001).

Chatham Island tūī are larger and heavier, have paler feathers and longer white throat feathers compared with mainland tūī (Dilks & Kearvell 1996; Dilks 2004; Ballance & Morris 2006). It is not known whether there are any behavioural differences between mainland and Chatham Island tūī, including their vocalisations. It was predicted that the larger overall mass of the Chatham Island subspecies might result in the production of lower frequency sounds (Pijanowski et al. 2011).

The aim of this study was to investigate differences in vocal characteristics between mainland tūī populations and Chatham Island tūī. Additionally, we predicted a lower syllabic diversity in Chatham Island tūī, resulting from geographic isolation, which presents tūī with a lesser scope for immigration and emigration as well as lower levels of male competition. We also predicted differences in frequency parameters between the mainland and the subspecies, due to morphological disparities and greater areas of closed habitats within the Chatham Islands. Acoustic adaptation theory predicts a greater percentage of trills would be produced by tūī on the mainland where there are more open areas of vegetation compared with the Chatham Islands study sites.

Methods

Seven sites, two in the Chatham Island group and five on the mainland, were included in this study. Location sizes, a habitat description, and a list of dominant plant species is provided for each in Table 1.

Chatham Islands

The two study sites on the Chatham Islands were the uninhabited and predator-free 219-ha Rangatira Island, or South East Island (44°34′ S, 176°17′ E), and the 40-ha predator-proof-fenced Ellen Elizabeth Preece Conservation Covenant (Caravan Bush; 44°28′ S 176°17′ E) (Table 1; Fig. 1a) on the east coast of Pitt Island. On both Rangatira and Pitt islands, flax (*Phormium tenax*), on which the tūī primarily feed during late spring and early summer, was flowering during the period of data collection. This ensured tūī were abundant. An estimated 45% of Rangatira, approximately 98 ha, is covered by forest (Nilsson et al. 1994). At Caravan Bush, all introduced mammals have been eradicated from the covenant apart from mice (*Mus musculus*).

Mainland sites

Sample sizes are reflective of the density of tūī at each of the five mainland sites (Table 1; Fig. 1b), at the time of recording. Sampling effort varied according to the size of each site, in order to record as many different individuals at each site as possible.

Tawharanui Regional Park

Tawharanui Regional Park, 90 km north of central Auckland, New Zealand, is a 588-ha 'mainland island' located on the Tawharanui Peninsula. A fence was installed to exclude mammal predators and the coastal park became virtually predator-free in 2004, except for the presence of mice, following a successful poisoning and trapping programme (Brunton et al. 2008). Tawharanui Regional Park consists of predominantly grazed pasture (Auckland Council 2009). At least 50% of the tūī population were banded between 2009 and 2012 (S. J. Wells unpubl. data 2013).

Table 1. A broad description of the tūī vocalisation study sites including habitat complexity and dominant vegetation.

Site name	Size (ha)	Predator status	Habitat complexity	Dominant species	References
Rangatira Island	219	Predator-free island	High	*Phormium tenax, Muehlenbeckia australis, Pteridium esculentum, Plagianthus regius chathamicus, Myoporum laetum, Olearia traversii, Myrsine chathamica, Coprosma chathamica, Melicytus chathamicus, Pseudopanax chathamicus, Macropiper excelsum, Ripogonum scandens*	Nilsson et al. 1994; Dilks 2004; Roberts et al. 2007
Caravan Bush, Pitt island	40	Predator-proof fence	High	*Rhopalostylis sapida, Phormium tenax, Muehlenbeckia australis,Pseudopanax chathamicus, Corynocarpus laevigatus, Corokia macrocarpa*	Miskelly & Emberson 2008
Tawharanui	588	Predator-proof fence	High	*Leptospermum scoparium, Metrosideros excelsa, Agathis australis, Vitex lucens, Beilschmiedia tarairi, Rhopalostylis sapida*	Auckland Council 2009; S. D. Hill pers. obs
Wenderholm	134	Predator-controlled	Medium	*Metrosideros excelsa, Beilschmiedia tarairi, Kunzea ericoides, Sophora microphylla, Corynocarpus laevigatus, Cordyline australis, Rhopalostylis sapida*	James & Clout 1996; Lovegrove et al. 2002
Ark in the Park, Waitakere	1100	Predator-controlled	High	*Dacrydium cupressinum, Agathis australis, Metrosideros robusta, Podocarpus totara, Dacrycarpus dacrydioides, Dicksonia spp., Kunzea ericoides*	Fraser & Hauber 2008; Derraik 2009
Maungatautari Southern Enclosure	65	Predator-proof fence	High	*Dacrydium cupressinum, Beilschmiedia tawa, Ixerba brexioides, Weinmannia racemosa, Quintinia serrata*	Watts et al. 2011
Pohangina Wetlands	7.2	Predator-controlled	Low	*Dacrycarpus dacrydioides, Podocarpus totara, Phormium tenax, Cordyline australis, Clianthus puniceus*	S. D. Hill pers. obs
Totara Reserve, Pohangina	340	Predator-controlled	Medium	*Podocarpus totara, Dacrydium cupressinum, Metrosideros robusta, Prumnopitys taxifolia, Dacrycarpus dacrydioides, Rhopalostylis sapida*	S. D. Hill pers. obs

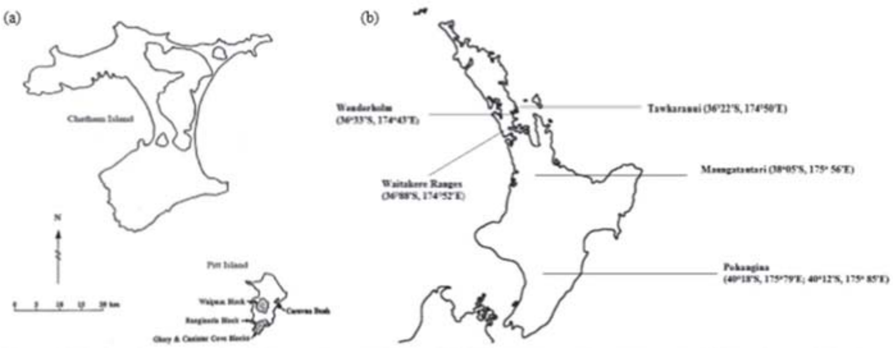


Figure 1. Map showing the location of Caravan Bush (44°28′ S, 176°17′ E) (a) (adapted from Walls et al. (2000), with permission from the Department of Conservation) and a map of New Zealand's North Island showing the locations of all of the mainland tūī study sites (b).

Wenderholm Regional Park

Wenderholm Regional Park, 46 km north of Auckland, is a 134-ha mainland reserve. The park is bounded by two tidal estuaries and consists of an 80-ha hilly forested headland. Wenderholm also consists of a partly forested spit of open coastal pastureland and consolidated sands and wetlands bordering one of the surrounding estuaries (Lovegrove et al. 2002).

Ark in the Park, Waitakere Ranges Regional Park

Ark in the Park, within the Waitakere Ranges Regional Park, approximately 30 km west of Auckland, is a conservation area consisting of 1100 ha of forest.

Maungatautari Ecological Island

Maungatautari Ecological Island, 37 km south-east of Hamilton, Waikato, New Zealand, is a 3400-ha mainland island and an extinct andesitic volcanic cone. The Southern Enclosure of Maungatautari is 65 ha and is where recording took place. The reserve is surrounded by farmland.

Pohangina, Manawatu

Two sites in the Pohangina Valley, 30 km north-east of Palmerston North, Manawatu, were chosen. The first was the Pohangina Wetlands, a 7.2-ha nature reserve surrounded by farmland. The second site was the 340-ha Totara Reserve Regional Park located adjacent to large areas of farmland.

Sound recordings

Data collection of tūī songs on both the Chatham Islands and the mainland was carried out during the tūī breeding season (October–January). This occurred in 2010/11 (both sites) and 2012/13 (mainland only). During sampling sessions we walked slowly one way along marked tracks to locate tūī, and at all sites we attempted to cover as much area as possible. When a tūī was located, recording was initiated and ended when the tūī either ceased singing or flew away. During each recording, the location, time, the band combination of the tūī (if applicable), behaviour, and song type were also recorded.

Recordings were collected from banded tūī whenever possible. For unbanded birds, only one individual was recorded at any one time to avoid recording the same bird twice in any given sampling location, hence no individual was used more than once in song analysis. During the breeding season, when both nests and flowering plants are being guarded, male tūī will remain strictly within their respective territories, which helped avoid pseudoreplication. Furthermore, the highly territorial and habitual nature of tūī (i.e. individual males only singing on particular perches, S. D. Hill pers. obs.) also minimised the chances of pseudoreplication. All songs included in the analysis were recorded between 0800 and 1400 hours at a distance of between 2 m and 5 m from the bird.

A Sony Portable High-Definition MiniDisc Recorder (Sony, Park Ridge, NJ, USA) and a Marantz PMD620 Solid-State Digital Recorder (Marantz, Kanagawa, Japan) were used to record songs, along with a Sennheiser ME67 shotgun long-range directional microphone (Sennheiser, Old Lyme, CT). The uncompressed MiniDisc sound files were converted to wave files using Sony SonicStage software (Sony, NJ, USA). All songs were recorded at a sampling frequency of 44.1 KHz and at a resolution of 16-bits. The frequency response range of the long-range microphone was 50 to 20 000 Hz. The song spectrograms were digitised and song variables measured using the Raven Pro 1.4 Beta Version software (Cornell Lab of Ornithology, Ithaca, NY, USA) (discrete fourier transform (DFT) = 256, Hann window, 2.9 ms, 50% frame overlap, bandwidth = 3dB).

Song variables

To compare the spectral characteristics of tūī vocalisations between all mainland sites and Chatham Island tūī, eight song variables were extracted from each tūī song for analysis (Table 2; main variables are depicted in Fig. 2). These variables have been employed in previous studies for characterising bird songs (Mennill & Rogers 2006; Hennin et al. 2009).

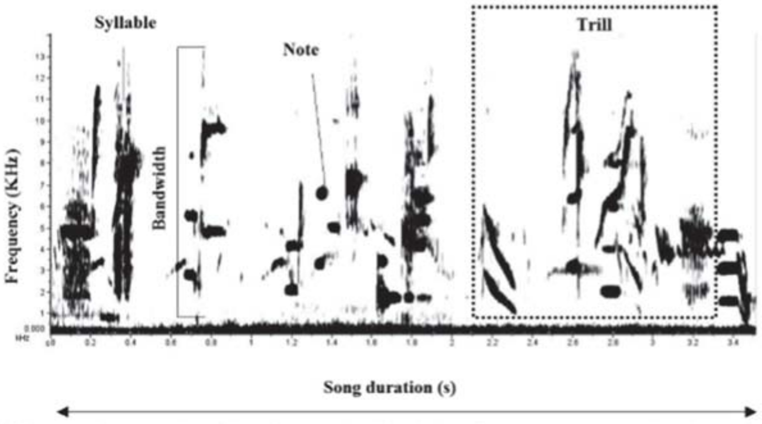


Figure 2. Diagrammatic representation of the variables employed in this study. The spectrogram presented here is part of a mainland male long-range tūī song.

Table 2. A summary and definition of the eight variables employed in this study to compare vocalisations between mainland and Chatham Island tūī (*Prosthemadera* spp.).

Song variable	Definition
Number of notes	The number of individual notes (fundamental sound units) per song
Song duration (seconds)	Time from the beginning of the first note to the end of the terminal note of each chosen song
Trill duration (seconds)	Cumulative total length of high-frequency modulation portions per song
Trill percentage	Percentage of each song that contains high-frequency modulation notes
Syllable diversity	The number of *different* sound unit complexes (syllables) per song. Syllables can be comprised of several notes
Maximum frequency (KHz)	The highest pitch produced in a song
Mean frequency (KHz)	Overall average pitch of sounds per song
Bandwidth (KHz)	The difference between maximum and minimum frequency

Comparison of song variables between mainland and Chatham Island tūī

Song variables described above were extracted from male long-range (broadcasting from a perch in a tree) songs and were explored using principal component analysis (PCA) with VARIMAX orthogonal rotation. This rotational procedure is designed to simplify the interpretation of calculated factors by changing the factor loadings, which represent correlations of each factor with the original variables, to be as maximally correlated with each principal component as possible, with the total amount of variance explained by the factors remaining unchanged (Hanson et al. 2002). Three variables, one from each principal component with factor loadings greater than 0.8, were selected for further Mann–Whitney–Wilcoxon tests, as the data distribution was not normal. The variable 'syllable diversity' was selected for further testing based on its significance noted by previous research (Boogert et al. 2008). Boxplots were created to visualise the data. Statistical tests were performed using SPSS software (version 20.0; SPSS, Chicago, IL, USA). All statistical tests were two-tailed and P values < 0.05 were interpreted as significant.

Analysis of similarity between mainland and Chatham Island tūī

The variables described above were examined for normality and the following were transformed to $\ln(x) + 0.01$ to remove skewness: number of notes, song duration, trill duration, trill percentage, syllable diversity, and maximum frequency. The variables from the mainland and the Chatham Islands were then examined by analysis of similarity (ANOSIM, 9999 permutations) following standardisation to z-scores (i.e. normalisation). ANOSIM is a non-parametric multivariate test based on, in this instance, Euclidean distance measure, using the rank order of dissimilarity values to test whether the overall song structure differed between the mainland and Chatham Island populations. ANOSIM was performed using PAST software (version 2.17b) (Hammer et al. 2001).

Results

Comparison of overall song structure between the two sites

There was no significant difference in the ranked distance within mainland sites (ANOSIM, $n = 61$; $R = -0.155$, $P = 0.982$, 9999 permutations) allowing data from all mainland sites to be pooled (Tawharanui: $n = 37$; Wenderholm: $n = 14$; Waitakere: $n = 3$; Maungatautari: $n = 3$; Pohangina: $n = 4$; Total: $n = 61$).

There was a significant difference in the ranked distance between mainland tūī and the Chatham Island population (ANOSIM, $n = 61, 44$; $R = 0.131$, $P < 0.001$, 9999 permutations).

Comparison of song variables between mainland and Chatham Island tūī

Principal component analysis with VARIMAX rotation was performed in male long-range songs between all mainland sites and Chatham Island tūī ($n = 61, 44$, Table 1; Fig. 3). The first three principal components (PCs) cumulatively explained 94.59% of the variance between the mainland and Chatham Island populations. PC1 was correlated with maximum frequency, mean frequency, and bandwidth. PC2 was correlated with the number of notes, song duration and syllable diversity, and PC3 with trill duration and trill percentage (Table 3).

The mean frequency of songs did not differ between the mainland and Chatham Island populations ($P = 0.885$). However, mainland tūī had a significantly greater syllable diversity ($P = 0.038$) and contained a higher percentage of trills ($P = 0.009$) than the Chatham Island population (Table 4; Fig. 4).

Discussion

Song variation

Consistent with our prediction, song spectral variables of male long-range 'broadcasting' songs differed between the mainland and Chatham Island tūī populations. Moreover, Chatham Island tūī had a significantly smaller percentage of song that contained trills in addition to a lower syllable diversity than their mainland conspecifics. These variables have been demonstrated as being sexually selected traits linked to male genetic diversity (van Buskirk 1997; Nowicki et al. 1998; Ballentine et al. 2004). With greater geographic isolation, Chatham Island tūī experience a lesser degree of emigration and immigration than mainland tūī (Dilks & Kearvell 1996; Department of Conservation 2001). The limited opportunities for dispersal in the Chatham Island subspecies may have contributed to lower genetic diversity. The observed differences in song characteristics from this population may be a reflection of this. However, further studies on genetic diversity in tūī are required to confirm such a relationship.

Contrary to our prediction, there were no significant differences in frequency parameters between the populations. Lower-frequency shifts attributable to habitat and morphological differences (Pijanowski et al. 2011) have been shown in past

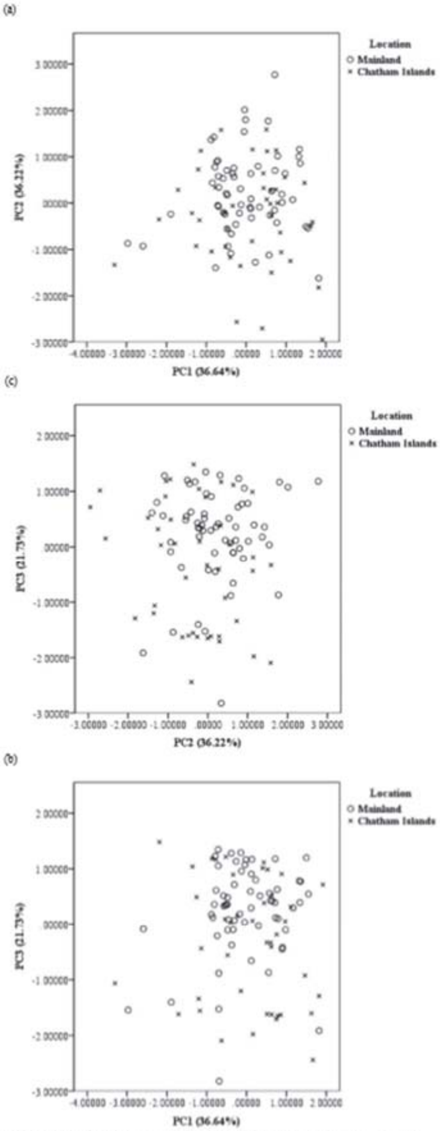


Figure 3. VARIMAX-rotated PCA (principal component analysis) scatterplots (PC1 and PC2 (a); PC1 and PC3 (b); PC2 and PC3 (c)), showing the principal component scores for each individual from mainland and Chatham Island tūī defined by PC1, PC2 and PC3, obtained from eight variables.

Table 3. Eigenvalues, variance explained and factor loadings of the eight variables following principal component (PC) analysis for male long-range songs between the mainland and Chatham Island tūī (n = 61, 44). Those variables with factor loading values greater than 0.8 are shown in bold.

	PC1	PC2	PC3
Eigenvalue	2.93	2.90	1.74
% of variance	36.64	36.22	21.73
Number of notes	0.08	**0.93**	0.18
Song duration	0.18	**0.91**	0.09
Trill duration	0.11	0.54	**0.82**
Trill percentage	0.01	0.06	**0.99**
Syllable diversity	0.16	**0.91**	0.17
Maximum frequency	**0.96**	0.17	0.09
Mean frequency	**0.99**	0.12	0.02
Bandwidth	**0.98**	0.13	0.02

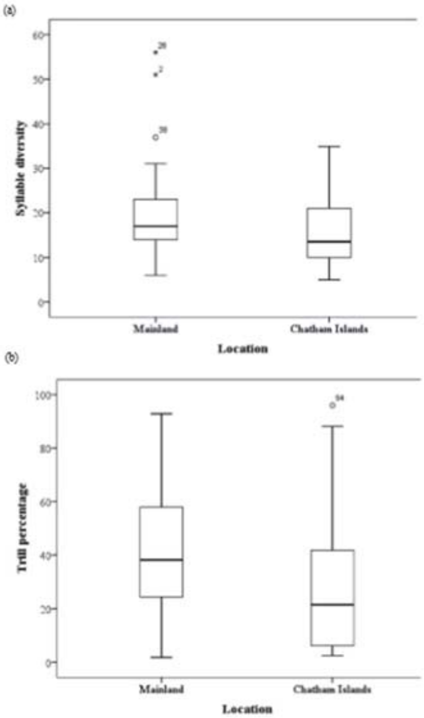


Figure 4. Boxplots of variables with statistically significant differences between the mainland and Chatham Island tūī populations in male, long-range songs. Syllable diversity (a), and percentage of song containing trills (b).

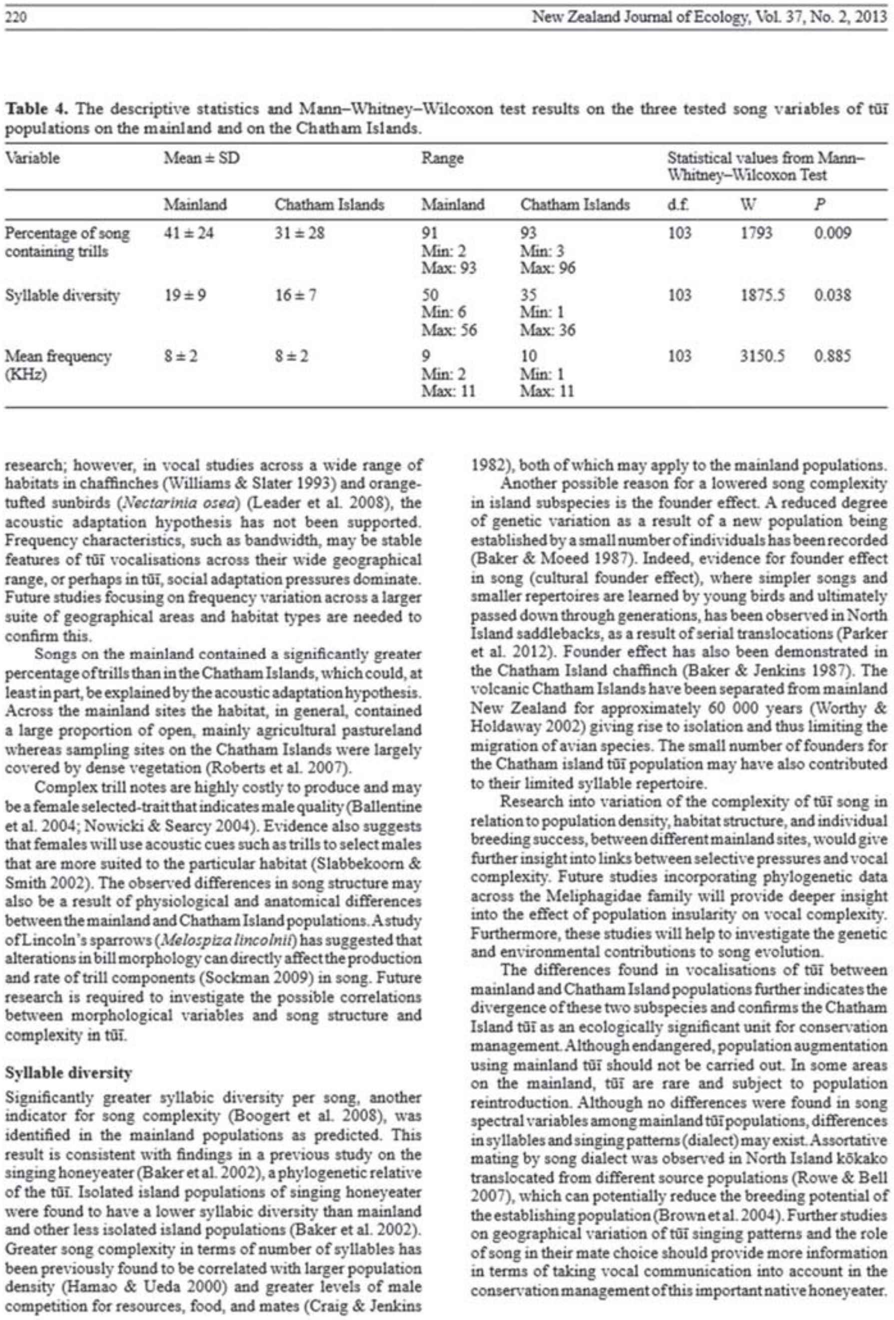


Table 4. The descriptive statistics and Mann–Whitney–Wilcoxon test results on the three tested song variables of tūī populations on the mainland and on the Chatham Islands.

Variable	Mean ± SD		Range		Statistical values from Mann–Whitney–Wilcoxon Test		
	Mainland	Chatham Islands	Mainland	Chatham Islands	d.f.	W	*P*
Percentage of song containing trills	41 ± 24	31 ± 28	91 Min: 2 Max: 93	93 Min: 3 Max: 96	103	1793	0.009
Syllable diversity	19 ± 9	16 ± 7	50 Min: 6 Max: 56	35 Min: 1 Max: 36	103	1875.5	0.038
Mean frequency (KHz)	8 ± 2	8 ± 2	9 Min: 2 Max: 11	10 Min: 1 Max: 11	103	3150.5	0.885

research; however, in vocal studies across a wide range of habitats in chaffinches (Williams & Slater 1993) and orange-tufted sunbirds (*Nectarinia osea*) (Leader et al. 2008), the acoustic adaptation hypothesis has not been supported. Frequency characteristics, such as bandwidth, may be stable features of tūī vocalisations across their wide geographical range, or perhaps in tūī, social adaptation pressures dominate. Future studies focusing on frequency variation across a larger suite of geographical areas and habitat types are needed to confirm this.

Songs on the mainland contained a significantly greater percentage of trills than in the Chatham Islands, which could, at least in part, be explained by the acoustic adaptation hypothesis. Across the mainland sites the habitat, in general, contained a large proportion of open, mainly agricultural pastureland whereas sampling sites on the Chatham Islands were largely covered by dense vegetation (Roberts et al. 2007).

Complex trill notes are highly costly to produce and may be a female selected-trait that indicates male quality (Ballentine et al. 2004; Nowicki & Searcy 2004). Evidence also suggests that females will use acoustic cues such as trills to select males that are more suited to the particular habitat (Slabbekoorn & Smith 2002). The observed differences in song structure may also be a result of physiological and anatomical differences between the mainland and Chatham Island populations. A study of Lincoln's sparrows (*Melospiza lincolnii*) has suggested that alterations in bill morphology can directly affect the production and rate of trill components (Sockman 2009) in song. Future research is required to investigate the possible correlations between morphological variables and song structure and complexity in tūī.

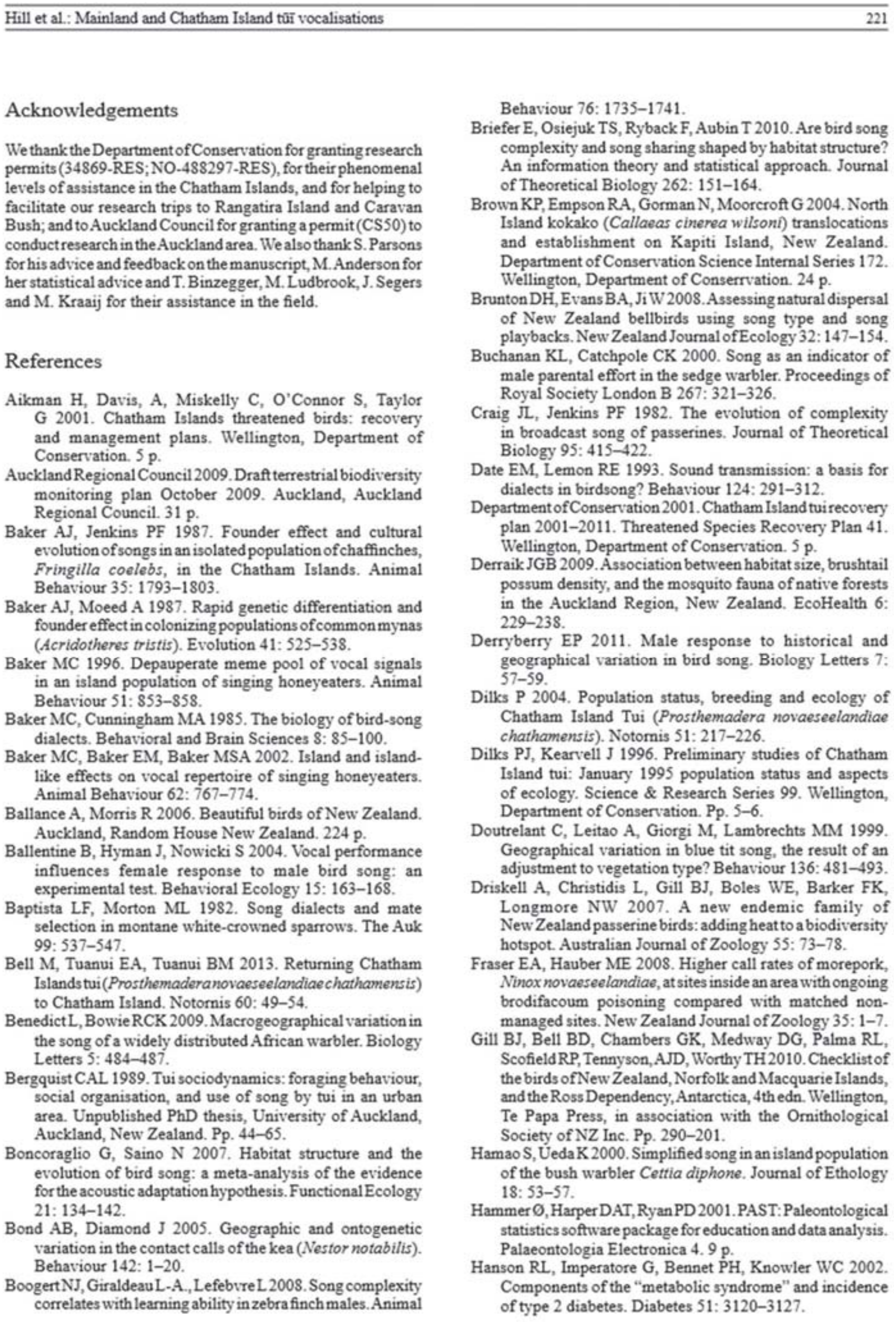
Syllable diversity

Significantly greater syllabic diversity per song, another indicator for song complexity (Boogert et al. 2008), was identified in the mainland populations as predicted. This result is consistent with findings in a previous study on the singing honeyeater (Baker et al. 2002), a phylogenetic relative of the tūī. Isolated island populations of singing honeyeater were found to have a lower syllabic diversity than mainland and other less isolated island populations (Baker et al. 2002). Greater song complexity in terms of number of syllables has been previously found to be correlated with larger population density (Hamao & Ueda 2000) and greater levels of male competition for resources, food, and mates (Craig & Jenkins 1982), both of which may apply to the mainland populations.

Another possible reason for a lowered song complexity in island subspecies is the founder effect. A reduced degree of genetic variation as a result of a new population being established by a small number of individuals has been recorded (Baker & Moeed 1987). Indeed, evidence for founder effect in song (cultural founder effect), where simpler songs and smaller repertoires are learned by young birds and ultimately passed down through generations, has been observed in North Island saddlebacks, as a result of serial translocations (Parker et al. 2012). Founder effect has also been demonstrated in the Chatham Island chaffinch (Baker & Jenkins 1987). The volcanic Chatham Islands have been separated from mainland New Zealand for approximately 60 000 years (Worthy & Holdaway 2002) giving rise to isolation and thus limiting the migration of avian species. The small number of founders for the Chatham island tūī population may have also contributed to their limited syllable repertoire.

Research into variation of the complexity of tūī song in relation to population density, habitat structure, and individual breeding success, between different mainland sites, would give further insight into links between selective pressures and vocal complexity. Future studies incorporating phylogenetic data across the Meliphagidae family will provide deeper insight into the effect of population insularity on vocal complexity. Furthermore, these studies will help to investigate the genetic and environmental contributions to song evolution.

The differences found in vocalisations of tūī between mainland and Chatham Island populations further indicates the divergence of these two subspecies and confirms the Chatham Island tūī as an ecologically significant unit for conservation management. Although endangered, population augmentation using mainland tūī should not be carried out. In some areas on the mainland, tūī are rare and subject to population reintroduction. Although no differences were found in song spectral variables among mainland tūī populations, differences in syllables and singing patterns (dialect) may exist. Assortative mating by song dialect was observed in North Island kōkako translocated from different source populations (Rowe & Bell 2007), which can potentially reduce the breeding potential of the establishing population (Brown et al. 2004). Further studies on geographical variation of tūī singing patterns and the role of song in their mate choice should provide more information in terms of taking vocal communication into account in the conservation management of this important native honeyeater.



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References

Aikman H, Davis, A, Miskelly C, O'Connor S, Taylor G 2001. Chatham Islands threatened birds: recovery and management plans. Wellington, Department of Conservation. 5 p.

Auckland Regional Council 2009. Draft terrestrial biodiversity monitoring plan October 2009. Auckland, Auckland Regional Council. 31 p.

Baker AJ, Jenkins PF 1987. Founder effect and cultural evolution of songs in an isolated population of chaffinches, *Fringilla coelebs*, in the Chatham Islands. Animal Behaviour 35: 1793–1803.

Baker AJ, Moeed A 1987. Rapid genetic differentiation and founder effect in colonizing populations of common mynas (*Acridotheres tristis*). Evolution 41: 525–538.

Baker MC 1996. Depauperate meme pool of vocal signals in an island population of singing honeyeaters. Animal Behaviour 51: 853–858.

Baker MC, Cunningham MA 1985. The biology of bird-song dialects. Behavioral and Brain Sciences 8: 85–100.

Baker MC, Baker EM, Baker MSA 2002. Island and island-like effects on vocal repertoire of singing honeyeaters. Animal Behaviour 62: 767–774.

Ballance A, Morris R 2006. Beautiful birds of New Zealand. Auckland, Random House New Zealand. 224 p.

Ballentine B, Hyman J, Nowicki S 2004. Vocal performance influences female response to male bird song: an experimental test. Behavioral Ecology 15: 163–168.

Baptista LF, Morton ML 1982. Song dialects and mate selection in montane white-crowned sparrows. The Auk 99: 537–547.

Bell M, Tuanui EA, Tuanui BM 2013. Returning Chatham Islands tui (*Prosthemadera novaeseelandiae chathamensis*) to Chatham Island. Notornis 60: 49–54.

Benedict L, Bowie RCK 2009. Macrogeographical variation in the song of a widely distributed African warbler. Biology Letters 5: 484–487.

Bergquist CAL 1989. Tui sociodynamics: foraging behaviour, social organisation, and use of song by tui in an urban area. Unpublished PhD thesis, University of Auckland, Auckland, New Zealand. Pp. 44–65.

Boncoraglio G, Saino N 2007. Habitat structure and the evolution of bird song: a meta-analysis of the evidence for the acoustic adaptation hypothesis. Functional Ecology 21: 134–142.

Bond AB, Diamond J 2005. Geographic and ontogenetic variation in the contact calls of the kea (*Nestor notabilis*). Behaviour 142: 1–20.

Boogert NJ, Giraldeau L-A., Lefebvre L 2008. Song complexity correlates with learning ability in zebra finch males. Animal Behaviour 76: 1735–1741.

Briefer E, Osiejuk TS, Ryback F, Aubin T 2010. Are bird song complexity and song sharing shaped by habitat structure? An information theory and statistical approach. Journal of Theoretical Biology 262: 151–164.

Brown KP, Empson RA, Gorman N, Moorcroft G 2004. North Island kokako (*Callaeas cinerea wilsoni*) translocations and establishment on Kapiti Island, New Zealand. Department of Conservation Science Internal Series 172. Wellington, Department of Conserrvation. 24 p.

Brunton DH, Evans BA, Ji W 2008. Assessing natural dispersal of New Zealand bellbirds using song type and song playbacks. New Zealand Journal of Ecology 32: 147–154.

Buchanan KL, Catchpole CK 2000. Song as an indicator of male parental effort in the sedge warbler. Proceedings of Royal Society London B 267: 321–326.

Craig JL, Jenkins PF 1982. The evolution of complexity in broadcast song of passerines. Journal of Theoretical Biology 95: 415–422.

Date EM, Lemon RE 1993. Sound transmission: a basis for dialects in birdsong? Behaviour 124: 291–312.

Department of Conservation 2001. Chatham Island tui recovery plan 2001–2011. Threatened Species Recovery Plan 41. Wellington, Department of Conservation. 5 p.

Derraik JGB 2009. Association between habitat size, brushtail possum density, and the mosquito fauna of native forests in the Auckland Region, New Zealand. EcoHealth 6: 229–238.

Derryberry EP 2011. Male response to historical and geographical variation in bird song. Biology Letters 7: 57–59.

Dilks P 2004. Population status, breeding and ecology of Chatham Island Tui (*Prosthemadera novaeseelandiae chathamensis*). Notornis 51: 217–226.

Dilks PJ, Kearvell J 1996. Preliminary studies of Chatham Island tui: January 1995 population status and aspects of ecology. Science & Research Series 99. Wellington, Department of Conservation. Pp. 5–6.

Doutrelant C, Leitao A, Giorgi M, Lambrechts MM 1999. Geographical variation in blue tit song, the result of an adjustment to vegetation type? Behaviour 136: 481–493.

Driskell A, Christidis L, Gill BJ, Boles WE, Barker FK, Longmore NW 2007. A new endemic family of New Zealand passerine birds: adding heat to a biodiversity hotspot. Australian Journal of Zoology 55: 73–78.

Fraser EA, Hauber ME 2008. Higher call rates of morepork, *Ninox novaeseelandiae*, at sites inside an area with ongoing brodifacoum poisoning compared with matched non-managed sites. New Zealand Journal of Zoology 35: 1–7.

Gill BJ, Bell BD, Chambers GK, Medway DG, Palma RL, Scofield RP, Tennyson, AJD, Worthy TH 2010. Checklist of the birds of New Zealand, Norfolk and Macquarie Islands, and the Ross Dependency, Antarctica, 4th edn. Wellington, Te Papa Press, in association with the Ornithological Society of NZ Inc. Pp. 290–201.

Hamao S, Ueda K 2000. Simplified song in an island population of the bush warbler *Cettia diphone*. Journal of Ethology 18: 53–57.

Hammer Ø, Harper DAT, Ryan PD 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4. 9 p.

Hanson RL, Imperatore G, Bennet PH, Knowler WC 2002. Components of the "metabolic syndrome" and incidence of type 2 diabetes. Diabetes 51: 3120–3127.

Hennin HL, Barker, NKS, Bradley DW, Mennill DJ 2009. Bachelor and paired male rufous-and-white wrens use different singing strategies. Behavioral Ecology and Sociobiology 64: 151–159.

James RE, Clout MN 1996. Nesting success of New Zealand pigeons (*Hemiphaga novaeseelandiae*) in response to a rat (*Rattus rattus*) poisoning programme at Wenderholm Regional Park. New Zealand Journal of Ecology 20: 45–51.

Kroodsma DE 2005. The singing life of birds: the art and science of listening to birdsong. Boston, Houghton Mifflin. 119 p.

Leader N, Wright J, Yom-Tov Y 2000. Microgeographic song dialects in the orange-tufted sunbird (*Nectarinia osea*). Behaviour 137: 1613–1627.

Leader N, Geffen E, Mokady O, Yom-Tov Y 2008. Song dialects do not restrict gene flow in an urban population of the orange-tufted sunbird, *Nectarinia osea*. Behavioral Ecology and Sociobiology 62: 1299–1305.

Lovegrove TG, Zeiler CH, Greene BS, Green BW, Gaastra R, MacArthur AD 2002. Alien plant and animal control and aspects of ecological restoration in a small 'mainland island': Wenderholm Regional Park, New Zealand. In: Veitch CR, Clout MN eds Turning the tide: the eradication of invasive species. Gland, IUCN SSC Invasive Species Specialist Group. Pp. 155–163.

Marler P, Tamura M 1962. Song "dialects" in three populations of white-crowned sparrows. Condor 64: 368–377.

Mennill DJ, Rogers AC 2006. Whip it good! Geographic consistency in male songs and variability in female songs of the duetting eastern whipbird *Psophodes olivaceus*. Journal of Avian Biology 37: 93–100.

Miskelly CM, Emberson RM 2008. Assessment of potential Chatham Island snipe habitat on Pitt Island. DOC Research & Development Series 298. Wellington, Department of Conservation. 29 p.

Miskelly CM, Dowding JE, Elliott GP, Hitchmough RA, Powlesland RG, Robertson HA, Sagar PM, Scofield RP, Taylor GA 2008. Conservation status of New Zealand birds, 2008. Notornis 55: 117–135.

Nemeth E, Dabelsteen T, Pedersen SB, Winkler H 2006. Rainforests as concert halls for birds: are reverberations improving sound transmission of long song elements? The Journal of the Acoustical Society of America 119: 620–626.

Nilsson RJ, Kennedy ES, West JA 1994. The birdlife of South East Island (Rangatira), Chatham Islands, New Zealand. Notornis 41 (Suppl.): S109–S125.

Nowicki S, Searcy WA 2004. Song function and the evolution of female preferences: Why birds sing, why brains matter. Annals of the New York Academy of Sciences 1016: 704–723.

Nowicki S, Peters S, Podos J 1998. Song learning, early nutrition and sexual selection in songbirds. American Zoologist 38: 179–190.

Parker KA, Anderson MJ, Jenkins PF, Brunton DH 2012. The effects of translocation-induced isolation and fragmentation on the cultural evolution of bird song. Ecology Letters 15: 778–785.

Payne RB 1978. Microgeographic variation in songs of splendid sunbirds *Nectarinia coccinigaster*: population phenetics, habitats and song dialects. Behaviour 65: 282–308.

Payne RB, Payne LL, Woods JL, Sorensen MD 2000. Imprinting and the origin of parasite–host species associations in brood-parasitic indigobirds, *Vidua chalybeata*. Animal Behaviour 59: 69–81.

Peters S, Searcy WA, Beecher MD, Nowicki S 2000. Geographic variation in the organization of song sparrow repertoires. The Auk 117: 936–942.

Pijanowski BC, Villanueva-Rivera LJ, Dumyahn SL, Farina A, Krause BL, Napoletano BM, Gage SH, Pieretti N 2011. Soundscape ecology: the science of sound in the landscape. BioScience 61: 203–216.

Pitocchelli J 2011. Macrogeographic variation in the song of the Mourning Warbler (*Oporornis philadelphia*). Canadian Journal of Zoology 89: 1027–1040.

Podos J, Warren PS 2007. The evolution of geographic variation in birdsong. Advances in the Study of Behavior 37: 403–458.

Ranjard L 2010. Computational biology of bird song evolution. Unpublished PhD thesis, University of Auckland, Auckland, New Zealand. Pp. 114–128.

Roberts CM, Duncan RP, Wilson K-J 2007. Burrowing seabirds affect forest regeneration, Rangatira Island, Chatham Islands, New Zealand. New Zealand Journal of Ecology 31: 208–222.

Rowe SJ, Bell BD 2007. The influence of geographic variation in song dialect on post-translocation pair formation in North Island kokako (*Callaeas cinerea wilsoni*). Notornis 54: 28–37.

Slabbekoorn H, Smith TB 2002. Bird song, ecology and speciation. Philosophical Transactions of the Royal Society of London B 357: 493–503.

Sockman KW 2009. Annual variation in vocal performance and its relationship with bill morphology in Lincoln's sparrows, *Melospiza lincolnii*. Animal Behaviour 77: 663–671.

Soma M, Garamszegi LZ 2010. Rethinking birdsong evolution: meta-analysis of the relationship between song complexity and reproductive success. Behavioral Ecology 22: 363–371.

Stewart AM, Craig JL 1985. Movements, status, access to nectar, and spatial organisation of the tui. New Zealand Journal of Zoology 12: 649–666.

Stilwell JD, Consoli CP 2012. Tectono-stratigraphic history of the Chatham Islands, SW Pacific—The emergence, flooding and reappearance of eastern 'Zealandia'. Proceedings of the Geologists' Association 123: 170–181.

Tu H-W, Severinghaus LL 2004. Geographic variation of the highly complex Hwamei (*Garrulax canorus*) songs. Zoological Studies 43: 629–640.

Van Buskirk J 1997. Independent evolution of song structure and note structure in American wood warblers. Proceedings of Royal Society London B 264: 755-761.

Walls G, Dilks P, Baird A 2000. Love them or lose them … Palm forests of Pitt Island and their wildlife. An ecological assessment of management needs, with comment from Pitt Islanders. Conservation Advisory Science Notes 283. Wellington, Department of Conservation. Pp. 4–13.

Watts CH, Armstrong DP, Innes J, Thornburrow D 2011. Dramatic increases in weta (Orthoptera) following mammal eradication on Maungatautari – evidence from pitfalls and tracking tunnels. New Zealand Journal of Ecology 35: 261–272.

White PJC 2012. Song characteristics vary clinally across an active colonization zone in Eurasian Nuthatches *Sitta europaea*. Bird Study 59: 296–302.

Williams JM, Slater PJB 1993. Does Chaffinch *Fringilla coelebs* song vary with the habitat in which it is sung? Ibis 135: 202–208.

Woodgate JL, Mariette MM, Bennett ATD, Griffith SC,

Buchanan KL 2012. Male song structure predicts reproductive success in a wild zebra finch population. Animal Behaviour 83: 773–781.
Worthy TH, Holdaway RN 2002. Lost world of the moa: prehistoric life of New Zealand. Bloomington, IN, Indiana University Press. 718 p.

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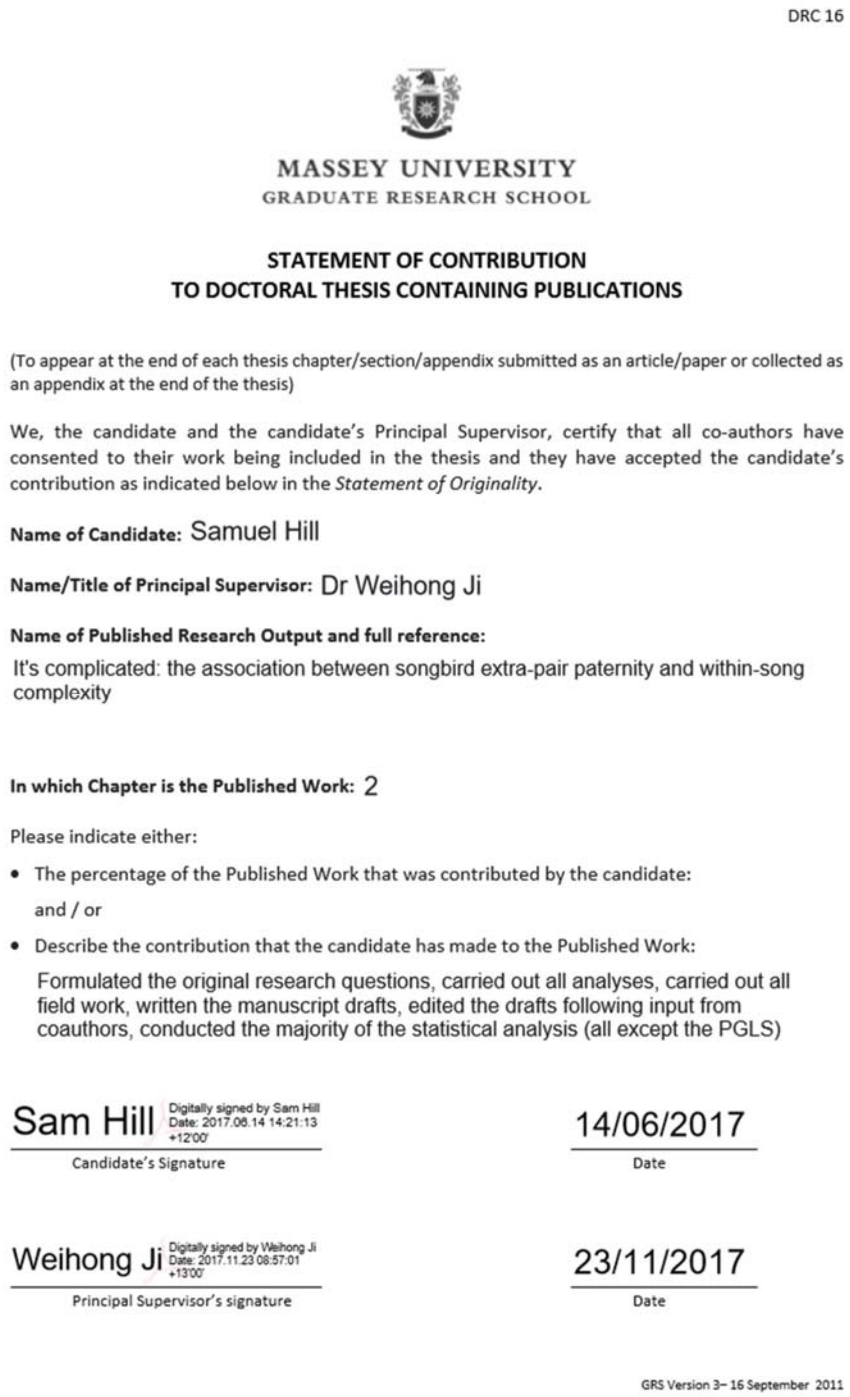
Appendix 4: Statement of contribution forms



DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Samuel Hill

Name/Title of Principal Supervisor: Dr Weihong Ji

Name of Published Research Output and full reference:

It's complicated: the association between songbird extra-pair paternity and within-song complexity

In which Chapter is the Published Work: 2

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Formulated the original research questions, carried out all analyses, carried out all field work, written the manuscript drafts, edited the drafts following input from coauthors, conducted the majority of the statistical analysis (all except the PGLS)

Sam Hill Digitally signed by Sam Hill Date: 2017.06.14 14:21:13 +12'00'
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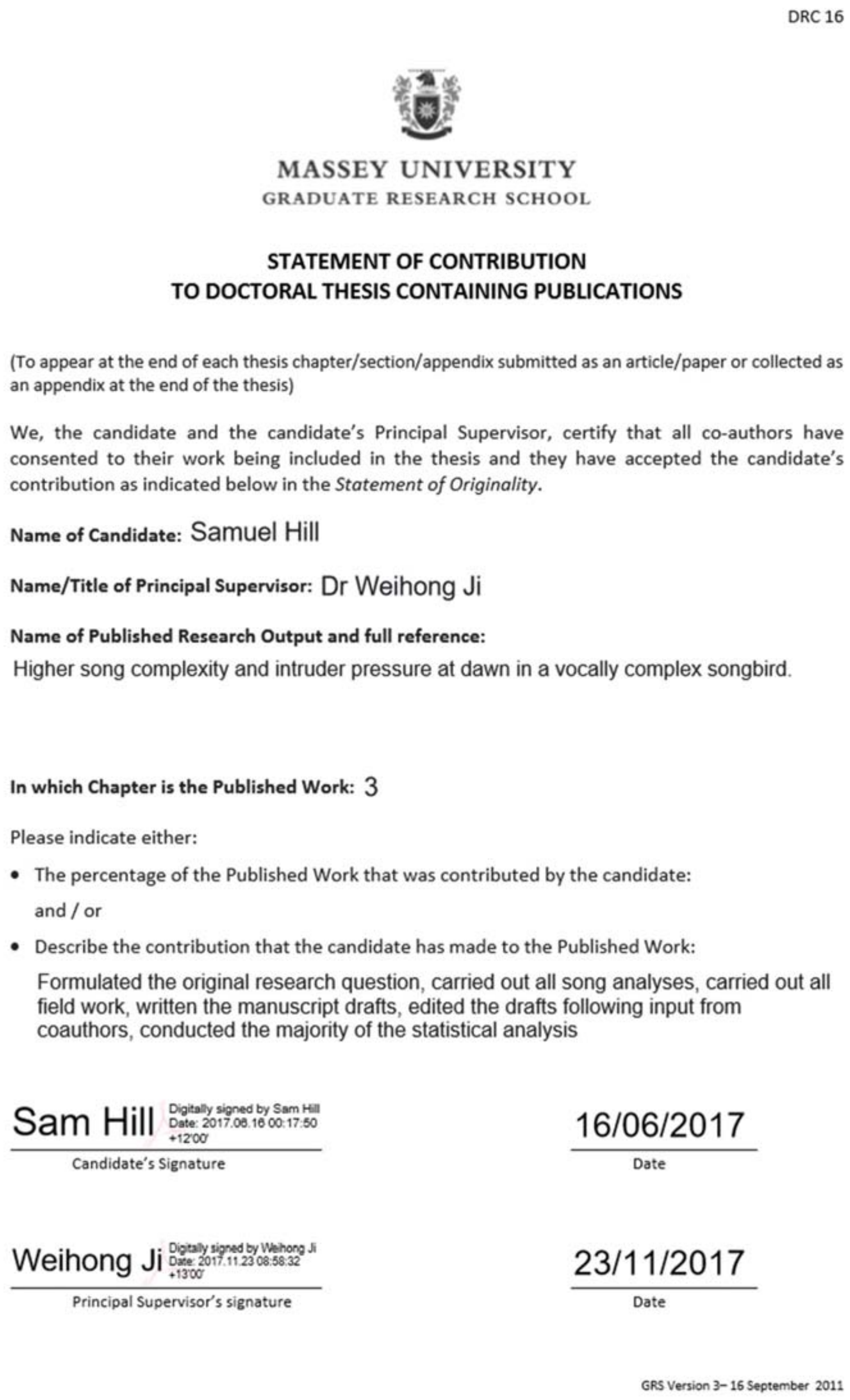

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MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Samuel Hill

Name/Title of Principal Supervisor: Dr Weihong Ji

Name of Published Research Output and full reference:

Higher song complexity and intruder pressure at dawn in a vocally complex songbird.

In which Chapter is the Published Work: 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Formulated the original research question, carried out all song analyses, carried out all field work, written the manuscript drafts, edited the drafts following input from coauthors, conducted the majority of the statistical analysis

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Date

Weihong Ji Digitally signed by Weihong Ji Date: 2017.11.23 08:58:32 +13'00'
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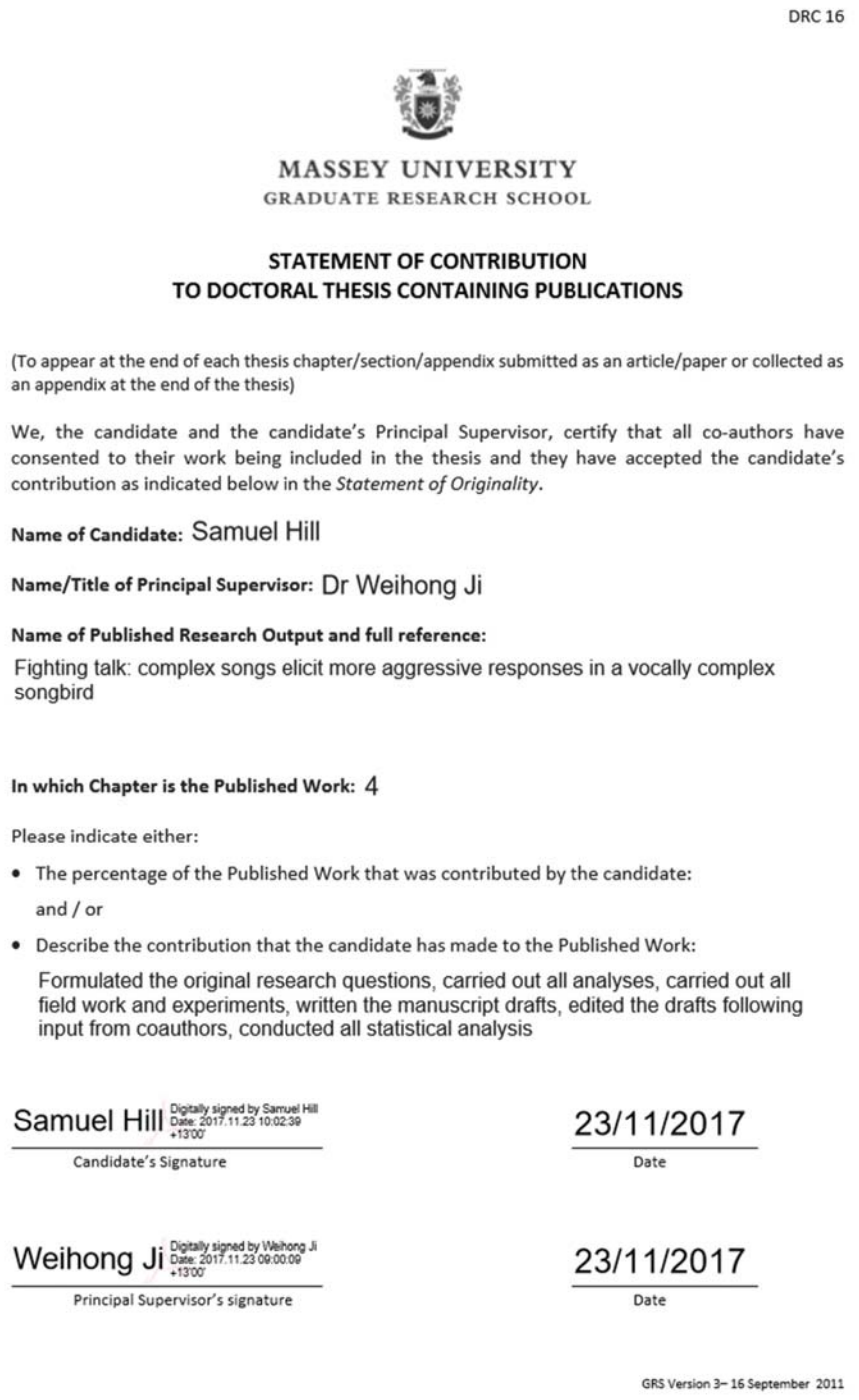

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Name of Candidate: Samuel Hill

Name/Title of Principal Supervisor: Dr Weihong Ji

Name of Published Research Output and full reference:

Fighting talk: complex songs elicit more aggressive responses in a vocally complex songbird

In which Chapter is the Published Work: 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Formulated the original research questions, carried out all analyses, carried out all field work and experiments, written the manuscript drafts, edited the drafts following input from coauthors, conducted all statistical analysis

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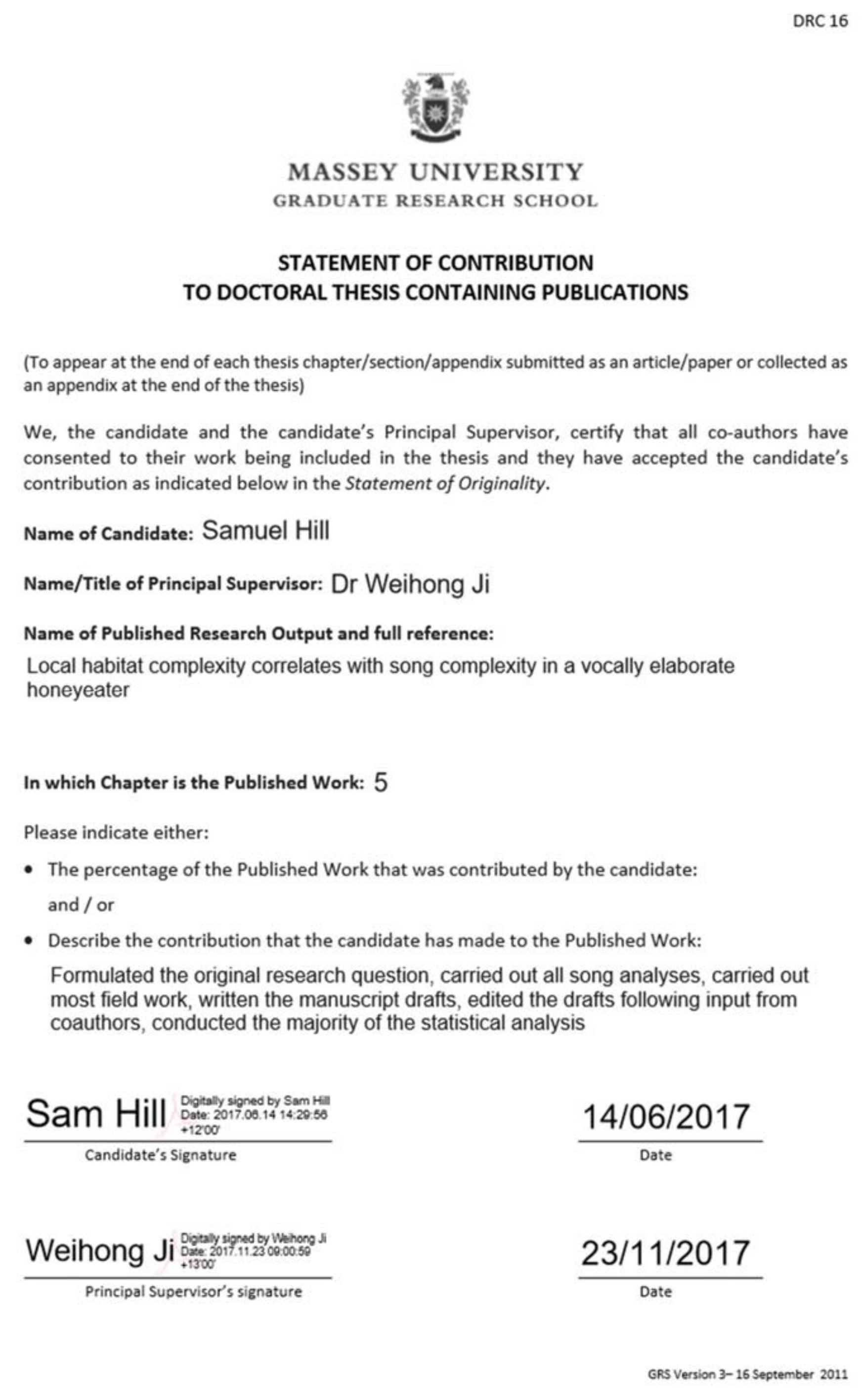

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Name of Candidate: Samuel Hill

Name/Title of Principal Supervisor: Dr Weihong Ji

Name of Published Research Output and full reference:

Local habitat complexity correlates with song complexity in a vocally elaborate honeyeater

In which Chapter is the Published Work: 5

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Formulated the original research question, carried out all song analyses, carried out most field work, written the manuscript drafts, edited the drafts following input from coauthors, conducted the majority of the statistical analysis

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Date: 2017.06.14 14:29:56
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Date: 2017.11.23 09:00:59
+13'00'

Principal Supervisor's signature

23/11/2017

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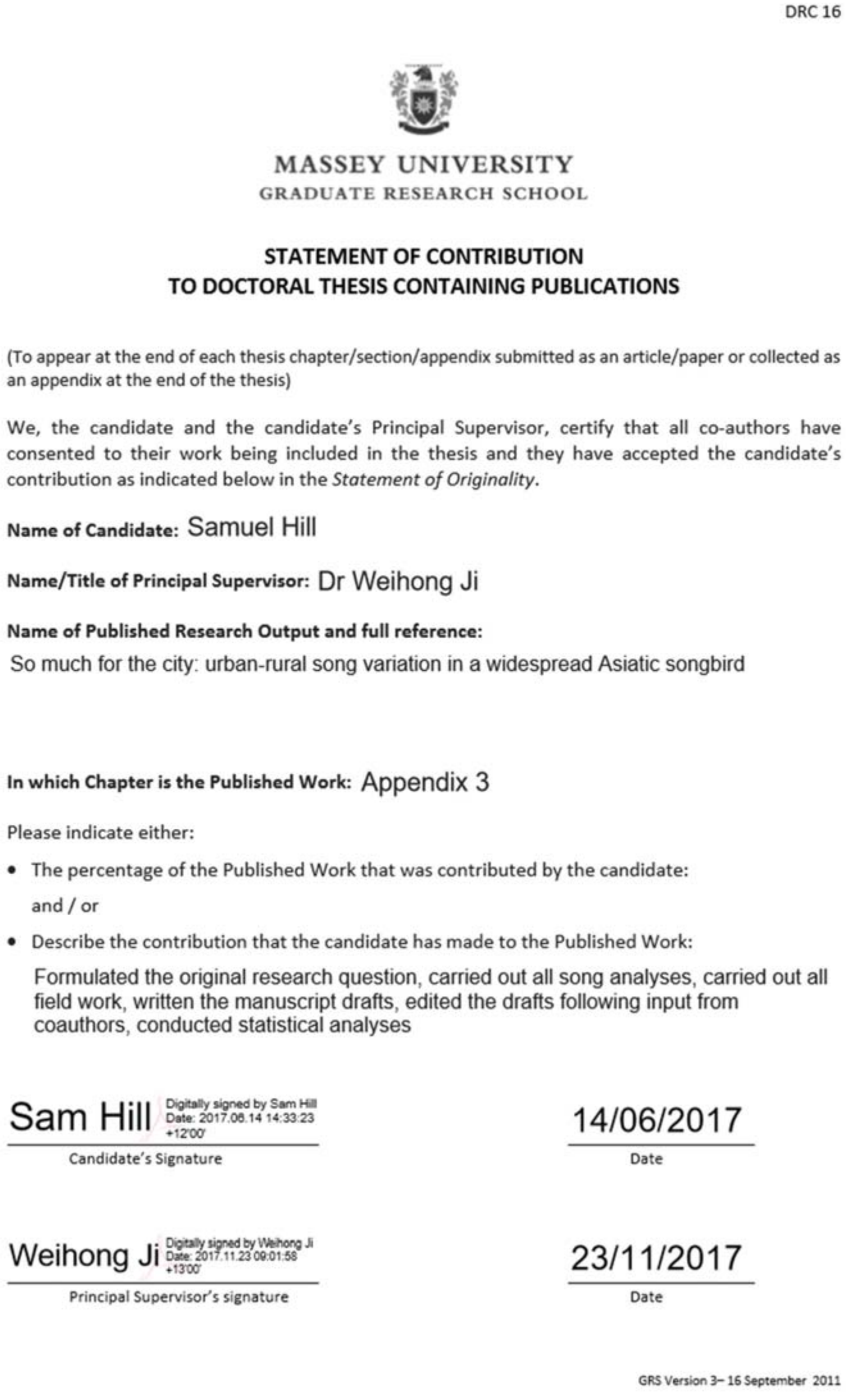

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Name of Candidate: Samuel Hill

Name/Title of Principal Supervisor: Dr Weihong Ji

Name of Published Research Output and full reference:

So much for the city: urban-rural song variation in a widespread Asiatic songbird

In which Chapter is the Published Work: Appendix 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Formulated the original research question, carried out all song analyses, carried out all field work, written the manuscript drafts, edited the drafts following input from coauthors, conducted statistical analyses

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Principal Supervisor's signature

23/11/2017
Date

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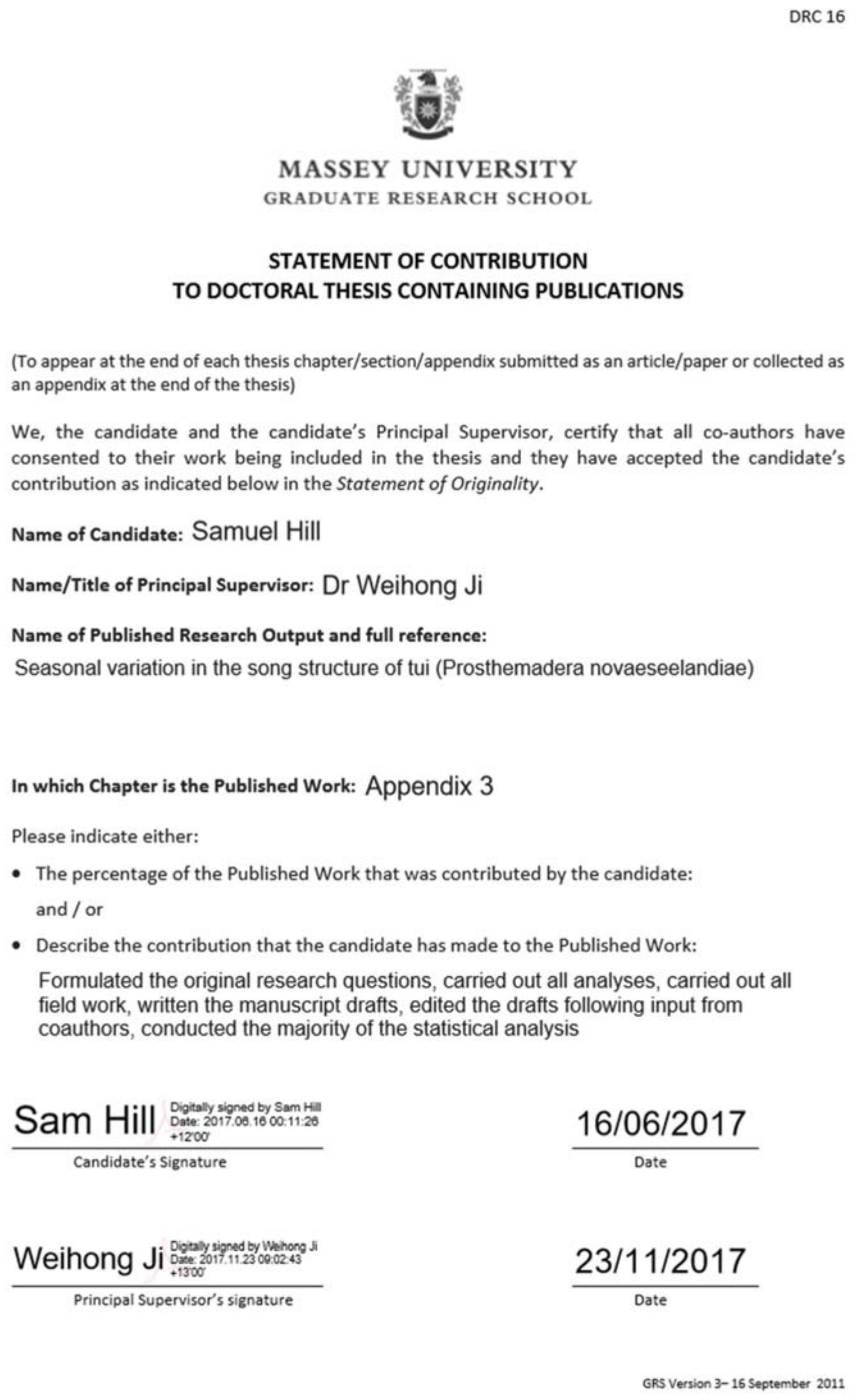

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Name of Candidate: Samuel Hill

Name/Title of Principal Supervisor: Dr Weihong Ji

Name of Published Research Output and full reference:

Seasonal variation in the song structure of tui (Prosthemadera novaeseelandiae)

In which Chapter is the Published Work: Appendix 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Formulated the original research questions, carried out all analyses, carried out all field work, written the manuscript drafts, edited the drafts following input from coauthors, conducted the majority of the statistical analysis

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Principal Supervisor's signature

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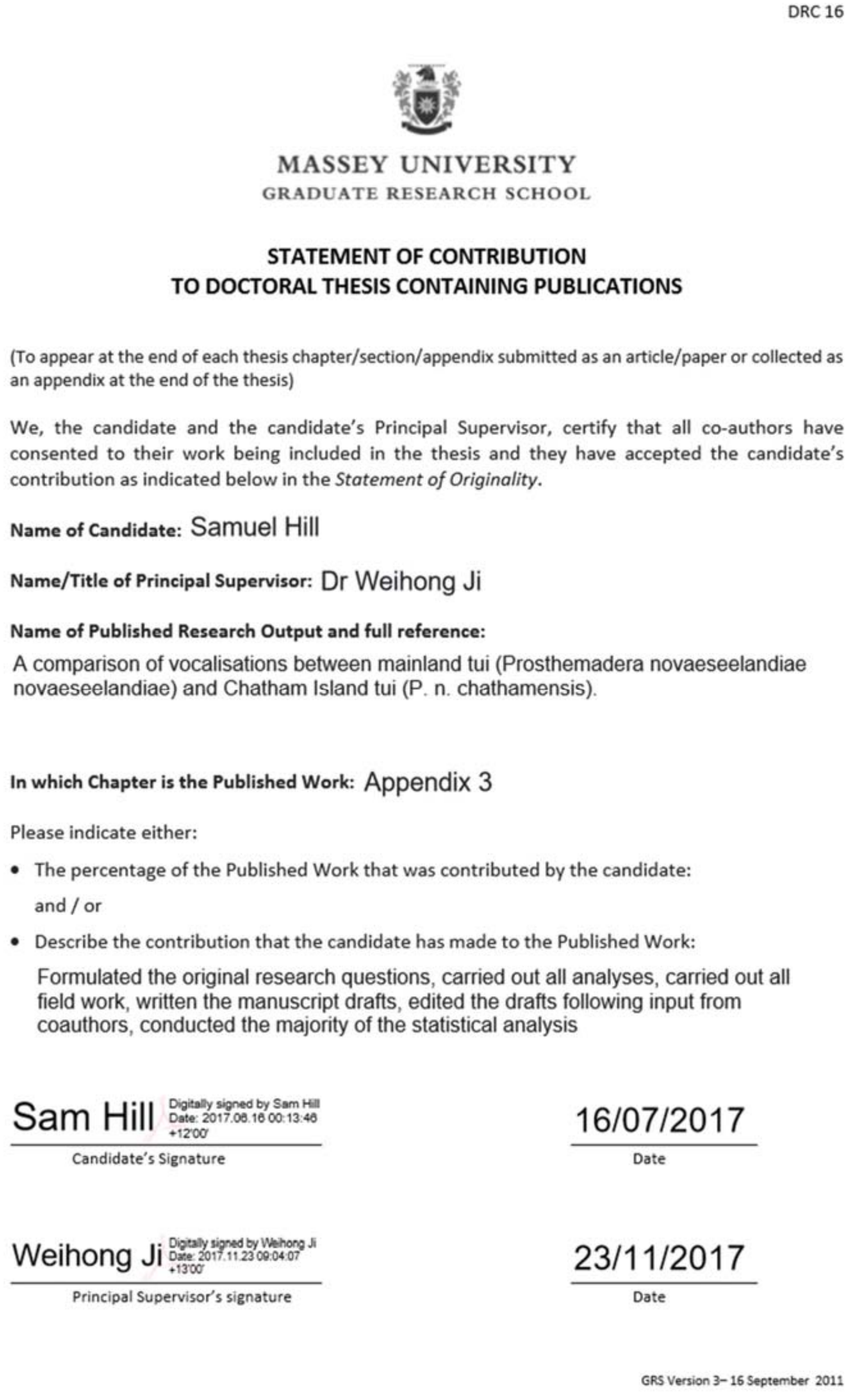

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Name of Candidate: Samuel Hill

Name/Title of Principal Supervisor: Dr Weihong Ji

Name of Published Research Output and full reference:

A comparison of vocalisations between mainland tui (Prosthemadera novaeseelandiae novaeseelandiae) and Chatham Island tui (P. n. chathamensis).

In which Chapter is the Published Work: Appendix 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Formulated the original research questions, carried out all analyses, carried out all field work, written the manuscript drafts, edited the drafts following input from coauthors, conducted the majority of the statistical analysis

Sam Hill Digitally signed by Sam Hill Date: 2017.08.16 00:13:46 +12'00'
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16/07/2017
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Principal Supervisor's signature

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Date

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