WELLINGTON GECKOS MEET WAIRARAPA GECKOS:

Hybridisation between two genetically and morphologically distinct populations of the New Zealand common gecko complex (*Hoplodactylus maculatus*)

A thesis presented in partial fulfilment of the requirements for the degree of

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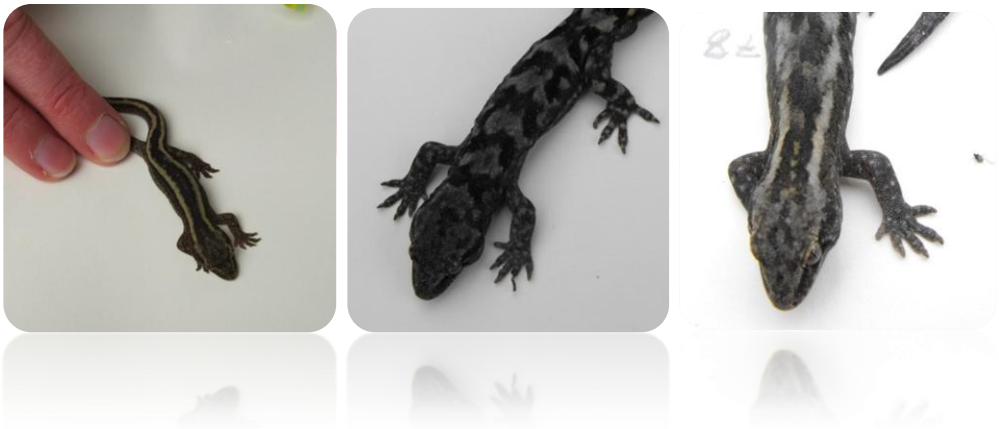

Abstract

The purpose of this study was to use molecular techniques and morphological measurements to set out to find whether a hybrid zone exists between two coastal populations of the common gecko (*Hoplodactylus maculatus*), on the Wellington south coast. I collected geckos from five sites in a coastal transect from the population of small geckos to the large geckos. Using four genetic loci, one mitochondrial (16S) and three nuclear (Rag-1, Rag-2, C-mos), I was able to determine that the coastal populations do have geneflow, however each population maintains some unique alleles. Morphological evidence reveals a significant difference in gecko sizes from Turakirae Head and those caught at Ocean Beach, separated by just 15 km. Adult geckos at Turakirae Head are on average 10mm smaller (snout-to-vent) than adult geckos at Ocean Beach, representing almost a doubling in average weight. The centre of the steep frequency clines of four characters is coincident and the widths are concordant. The narrower morphological clines indicate stronger selection on the size of the gecko, than on genetic loci.

DEDICATION

Dedicated to my fiancé

Adam Sullivan

For his wonderful support and encouragement

Acknowledgements

There have been a number of people who have helped in some way on this project and I am thankful to them all. My fiancé, Adam who went out on most field trips gecko sampling through some of the extreme weather and disappointing weekends. Also to my field assistants Christina, Shane, Shaun, Kayla, and Lewis who also helped collect samples. Also thanks to Sophie for some PCR work in the Lab and Trish for her knowledge with primers.

To Mary Morgan-Richards and Rod Hitchmough for their guidance and supervision of the project, which without would not have got so far.

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Contents

CONTENTS OF FIGURES

Contents of Tables

Introduction

Hybrid Zones

Hybridisation is the mating and production of offspring between individuals from genetically distinct populations (Harrison 1993). Hybrid zones form between two populations and/or species at the point of contact of their ranges. Many studies have looked at hybrid zones and selection against hybrids within the zone (Barton & Hewitt 1985; Moore 1977; Bronson et al 2003). The position and width of hybrid zones are usually stable over many generations, due to equilibrium between the ability of the taxon to disperse and the selective disadvantage suffered by the hybrid offspring (Barton & Hewitt 1985). Further stability is ensured when zones lie in density troughs (Barton 1979) or on ecotones (Moore 1977). Most hybrid zones involve secondary contact of populations that have diverged in isolation. Some hybrid zones effectively act as a barrier for speciation by allowing limited gene flow between the populations when hybrids backcross to parentals, and keeping populations genetically connected. However, separation of the two populations can be maintained by selection against the hybrids in a narrow contact zone and selection can maintain populations or species as distinct even when there is some gene flow (Alexandrino et al 2005). A narrow zone forms when hybrids are much less successful than the parental taxa (selective disadvantage against hybrids), for example if hybrids are sterile or suffer more from parasites. Alexandrino et al (2005) noted that there was a strong selection against hybrids in *Ensatina* (salamanders) at a hybrid zone, and that this was most likely due to a postzygotic mechanism; that reduce hybrid fitness compared to the parental fitness in this study. A wide zone forms where hybrids are as fit as parentals and this leads to the homogenising of populations and the eventual loss of a distinct zone of contact

(Johansson et al 2008).

Hybrid zones can be studied with any characters that distinguish the populations or species such as size, colour, behaviour, flowering time, chromosomes, enzymes, or DNA. Molecular markers are particularly useful in measuring hybrid zones and to detect dispersal of hybrids (Berry et al 2004). Mitochondrial DNA (mtDNA) is good to use as it is maternally inherited and non-recombinant with a rapid rate of evolution (Godinho et al 2006). However, because mtDNA is transmitted only by the maternal line it only provides one-sided information (Godinho et al 2006). Nuclear loci are useful in obtaining evidence of biparental gene flow and many single copy nuclear genes can be amplified from reptiles using universal primers (Gamble e al 2008; Godinho 2006, Vidal & Hedges 2004). For example, *C-mos* is a single coding exon of approximately 2 kb in the nuclear DNA that is present in all vertebrates (Godinho et al 2006). But any loci that show allele frequency differences among populations can be used to estimate gene flow. The three nuclear loci used in this study were all single copy genes and only partial genes were used.

There have been many studies looking at hybridisation in birds, toads and insects. In New Zealand there have been a few hybridisation studies, such as between the Auckland and Wellington tree weta, Grey and Mallard ducks, and Black and Pied stilts (Morgan-Richards et al 2009). It is common for evidence from morphology to tell a different story from that told by genetics. Sattler and Braun (2000) noted that previous morphological studies on a contact zone between Black-capped and Carolina chickadees found evidence of hybridisation while some studies found none. It was their study using genetic markers that showed hybridisation occurred frequently. This study showed the importance of using genetics in studies of species that may be cryptic to some extent.

The difference between morphology and genetics may be due to morphology usually being under selection, and many genetic differences being neutral. One method that is used to study hybrid zones is the use of hybrid clines (Leache and Cole, 2007). Often characters are scored using a hybrid index from 0-1 and/or principle component analysis or simple allele frequencies are used. This information can then be put into a hybrid cline that will indicate where the centre of the hybrid zone is located and estimate the zone width. Leache and Cole (2007) conducted a study on Fence lizards, *Sceloporus undulatus* complex, in the USA. They focused on a contact zone in eastern Arizona where two mtDNA lineages meet at an ecotone between grassland and woodland habitats. Northern and southern lineages differ in size, colouration, and squamation. Four characters were used for cline analysis including mtDNA, chromosomes, morphology and colour pattern. The results showed that three of four clines were coincident (Cline centre in the same place), but the mtDNA cline centre was shifted south from the other clines. The widths of these clines differed drastically and ranged from 1.5km-89km, thus they are nonconcordant. They suggested that the fence lizard contact zone has multiple processes working on it.

Species status

What is a species? This question is a dominant one in evolutionary biology of species formation. It has sparked great controversy and debate about the best way to differentiate species from each other. From the beginning species were considered based on their morphology alone, which would separate any morphological variant within a population into a different species as well as geographic populations (Bock 2004). As the understanding of genetics and speciation progressed it became clear that most populations and species had different varieties within populations as well as between

populations. One of the most common and popular concepts formed to try and define species was the biological species concept of Mayr (1942), which states that species are groups of individuals actually or potentially interbreeding natural populations which are reproductively isolated from other such groups. The understanding of species is important in understanding speciation including the hybridisation process and its consequences. Speciation is the formation of new species and hybridisation can have some positive or negative effects on this process. Previously hybridisation was thought of as an evolutionary dead end where hybrids were selected against by natural selection to prevent the loss of diversity (Arnold 1997). The hybridisation of two different species may introduce new genes and characters into a population that are better adapted to the environment for survival and overall fecundity, called hybrid vigour. On the other hand the complete opposite could occur where hybridisation may cause a homogenising effect of the two breeding species reducing the diversity between them.

It has been suggested by de Queiroz (1998) (quoted in Wiens & Servedio 2000) that the large number of species concepts agree on what a species is for sexual organisms, that is a species is a lineage which is unified primarily by sexual reproduction or gene flow among its constituent parts. If we use this as grounds to determining a species then gene flow is an important component in recognising and delimitating species, and hybridisation (the successful mating between two individuals) results in gene flow. A full understanding of the degree of gene flow between distinctive populations is essential for making decisions about species status. As conservation efforts are directed towards maintaining biodiversity (usually at the species level) it is extremely important that taxonomy takes into account the level of hybridisation between potential species and its probable long term consequences.

At the contact zone of distinct population ranges a number of things can potentially happen. Two species may mate and produce fertile hybrids resulting in gene flow between species, or two species may not mate and just coexist in the same habitat. The biological species concept states that two species cannot interbreed (Mayr 1942). Some interesting questions arise at a hybrid zone 1) if two "species" meet and mate does this mean they are actually the same species? 2) If they were separate can they now merge into one? 3) How well do hybrids do in terms of fitness compare to the parent species? In order to decide whether the populations are distinct enough to be considered separate species a number of features can be examined. Selection against hybrids (endogenous selection) is expected to result in characteristic genetic patterns (Kruuk et al 1999). For example, linkage of loci would be detected, as the allele combinations in parental populations (under possible selection) would result in significant linkage disequilibrium even when loci are not physically linked (e.g. on the same chromosome). Selection against hybrids would also be expected to result in a higher rate of heterozygosity in young geckos compared to adult geckos, and deviations from those expected by Hardy-Weinberg equilibrium. In contrast a species may diverge from another by selection for adaptations to a particular environment, a process known as exogenous selection (Kruuk 1999; Gee 2004). Ecological adaptation might result in ecotypes that differed only at loci under selection.

New Zealand Geckos

Geckos (Reptilia: Diplodactylidae) are a distinctive group of reptiles with a worldwide distribution (Robb 1980). The two genera of New Zealand geckos are monophyletic, with their closest relatives in Australia (Neilsen at al 2007). The species of *Naultinus* are predominantly diurnal, arboreal and usually bright green. The *Hoplodactylus* group

is usually nocturnal and found in a variety of habitats ranging from arboreal to ground dwelling and alpine to coast (Hitchmough 1997). Geckos are mostly small with some growing up to 35-40 cm (Robb 1980). There are about 17 described species of geckos in New Zealand (Hitchmough 1997), but this number continues to rise as genetic studies reveal cryptic species.

The common gecko (*Hoplodactylus maculatus*) (Gray 1843) is grey/brown in colour with a variety of different markings that run across the body (Whitaker 1996). It is a small to medium sized gecko with size variation among populations, for example *H. maculatus* from coastal populations are often smaller then inland individuals (Hitchmough 1997). The common gecko, widespread throughout most of New Zealand, is now known to be a complex of at least 12 species (Hitchmough 1997). There is considerable morphological variation within the *Hoplodactylus maculatus* complex, but only the use of genetic information has allowed the identification of this species diversity. Hitchmough describes the *Hoplodactylus maculatus* gecko species in greater detail (Hitchmough In prep). My research will focus on two populations of *Hoplodactylus maculatus* from the south coast of the North Island that are recognized by Hitchmough (1997) as conspecific.

Within the distribution of *Hoplodactylus maculatus* species complex are a series of monophyletic clades (Hitchmough 1997). The Northern group includes four species: *H. maculatus* and three tag species (*H*. "Mount Arthur", *H*. "Kaikouras", *H*. "Marlborough mini"). This Northern clade is found in the North Island and the top of the South Island around Nelson. The species *H. maculatus* (as referred to by Hitchmough 1997) is restricted to the North Island, including around Wellington and Wairarapa regions and lives in a variety of habitats including forest, scrub and grassland. *Hoplodactylus*

maculatus can be found under logs, rocks and debris and shingle banks (Robb 1980) and is more commonly found on the ground, being one of the less arboreal species (Whitaker 1996). Individuals from coastal areas may be bluish-grey in colour, with darker streaks on the dorsal surface (Robb 1980). They are nocturnal but may be seen sun-basking (Whitaker 1996). Coastal populations of the *Hoplodactylus maculatus* species complex are often smaller than inland sister groups, suggesting body size selection favours smaller animals in coastal habitats, where densities are often higher. Some coastal populations show sexual size dimorphism (with males larger than females; Whitaker 1982).

There are external morphological differences between female and male *Hoplodactylus maculatus* individuals. Males have 1-2 enlarged blunt scales on each side of the base of the tail (Robb 1980); and a broad patch of preanal and femoral pores along the underside of the thighs (Whitaker 1996) which contain glands (Robb 1980). The young are born mid-late summer (Robb 1980). Importantly the tail of this species is regenerated (Robb 1980), which is a defence mechanism and useful for our study purposes.

From previous study it has been observed by Whitaker (1987) that the geckos found on the coast are a lot smaller and more abundant than the geckos found further inland. This idea sparked this study looking at whether these different populations hybridise and therefore share genes and are still one species or whether they do not hybridise and are actually speciating into different species. To look at the differences between populations and seeing whether they are in the process of diverging we look at hybrid zones between the two populations that show the most difference.

Initial genetic studies using nuclear markers and mtDNA sequencing (Hitchmough

1997; Nielsen et al 2007) have found relatively high levels of genetic diversity within *Hoplodactylus maculatus*. Populations on the south coast of Wellington were found to be genetically distinct from geckos in Wairarapa at just a few allozyme loci (Hitchmough 1997). Hybridisation is thought to occur between these two populations which are recognized as members of the same species; *Hoplodactylus maculatus*. Morphological differences between these southern North Island populations have also been noted (Rod Hitchmough per com). The Wairarapa geckos sampled were larger than the coastal Wellington geckos, and sexual size dimorphism was limited to the coastal locations (Whitaker 1982).

My research will address five questions, which focus on the hypothesis that the Wellington coast and Wairarapa gecko populations might be cryptic species.

1) Using morphological and DNA sequences can one distinguish *Hoplodactylus maculatus* individuals from the Wellington South coast from those in Wairarapa?

2) Do these populations hybridise?

3) How wide is the hybrid zone?

4) Do the morphological and genetic (mtDNA and nuclear markers) zones have the same centres and similar widths?

5) Is this hybrid zone an effective barrier allowing speciation to occur?

Methods

Study site

The study site was located on the coast of Wellington starting from Turakirae Head and heading South-West towards Ocean Beach in Wairarapa (Figure 1). The two extreme populations that were thought to be the most morphologically different are shown in the red circles on the map below. Three more locations were sampled between these two extreme populations, approximately 3km apart. For an inland representative, samples were provided by Rod Hitchmough from his study of geckos (PhD unpublished thesis 1997) from Lake Pounui.

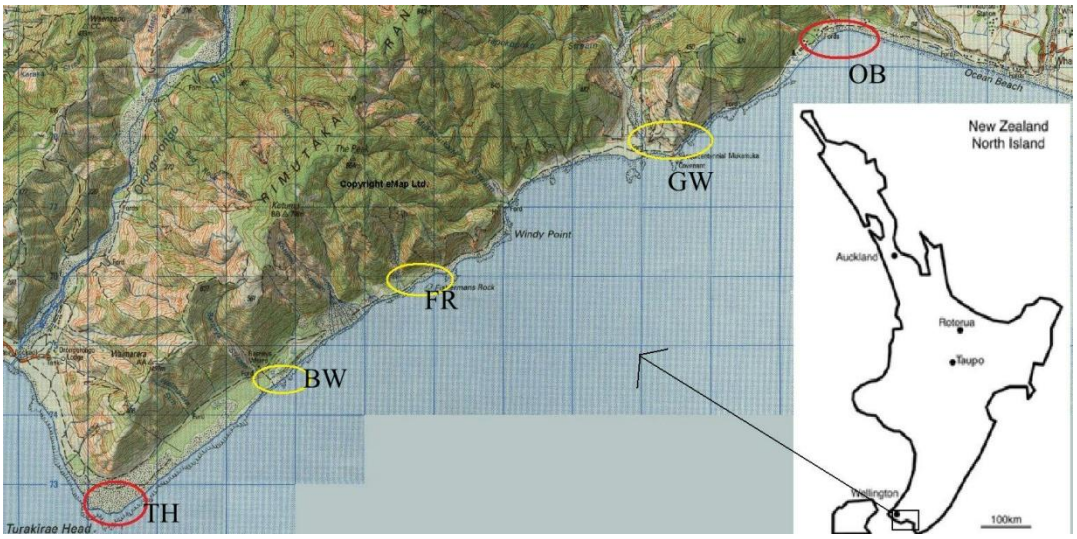


Figure 1, Map of Study site where geckos were collected from. The red circles indicate the two extreme populations at Turakirae Head (TH) and Ocean Beach (OB). Yellow circles are other populations sampled; Barneys Whare (BW), Fishermans Rock (FR) and Gateway (GW).

The locations were all within 20 meters of the shoreline, but they differed in habitat. Turakirae Head (TH) was fairly flat with large boulders and lots of small rock piles. The main vegetation was *Muehlenbeckia complexa* shrubs which are ideal for geckos to hide under (Whitaker 1982). The area was located close to Orongoronga station and there

were a number of sheep wandering around the area, as well as people. Barneys Whare (BW) is the next location and is a historic site. Around the cabin are lots of rocky places with *M. complexa* shrubs. The area is also close to some tall trees providing height and shelter from the sun. The third site is located at Fishermans rock (FR). This site is barren and the rock piles were within farmed grassland where sheep and cattle graze. The patch that housed the geckos caught for the study was small and located at the bottom of a hill. There were fewer shrubs there for protection. The next site is called Gateway (GW), and got its name due to a gate located across a path. This area was mostly steep hills with lots of rocks and almost scree like. The geckos were commonly found under reasonably sized rocks adjacent to the tall grass and the few shrubs. This site spanned the longest distance due to the sparsely populated spots ideal for geckos or gecko hunting. The last site is at Ocean beach (OB). This spot took the longest to find geckos suggesting that the population is less dense as they were able to elude capture due to the presence of human activity all year round. These geckos were found mostly in rocky areas in dried river beds. Later in the year they were found up to 20 cm underground in sandy and rocky areas next to shrubs or small trees. The coastal area around the study sites have been under some major geological influences. The particularly important geological activities that have altered the landscape are the southern North Island uplift and outwash from rivers. The Rimutaka Ranges which overshadow the study site are made up of Greywacke (Lee & Begg 2002). At the end of the Rimutakas at Turakirae Heads the area is dominated by beach and marginal marine terrace deposits consisting of marine gravels, sand, mud and the beach ridges (Lee & Begg 2002). Turakirae Heads is well known for its beach ridges which can be used to estimate dates of earthquakes that have raised the land. The latest beach ridge is about

2.7 meters from the current sea level. This rise was due to the 1855 Wairarapa fault earthquake that affected much of the region (McSaveney et al 2006).

Morphological Sampling

The common gecko was more easily found under medium sized rocks in rocky areas, or hiding under shrubs such as *Muehlenbeckia* that grows over rock piles. They were best caught when weather was still cool as they were less active due to their exothermic properties. At each site I aimed to collect 30 geckos at random. The first 20 geckos were included in both the genetic and the morphological study; the last 10 geckos from each site were used only for morphology. At two locations the full sample of 30 geckos were not obtained (Ocean Beach (n=28) and Gateway (n=25)). All geckos were caught between October 2008 and June 2009, except for 13 geckos with code name RAH caught by Rodney Hitchmough, which were collected prior to 1997 (these 13 geckos were included in the genetic analysis but not the morphological study).

Each gecko caught was placed in a zip lock plastic bag for measuring, and all measurements were made by one person (JF). The geckos were weighed using mini scales (Tanita Model 1479V). Quantitative external morphological measurements taken were; snout-vent length (SV), head width (HW), snout-ear length (SE) and head height (HH). Morphological characteristics recorded were; sex, eye colour, whether they were an adult or juvenile and had a damaged tail at catching or not. Using a hand lens the number of lamellae on the 4^{th} toe of the hind foot and external mites were counted and recorded. All lamellae, partial and whole, were counted from the base of the toe up to the tiny knob close to the toe nail. This knob was not actually included in the count however. Each gecko also had photos taken to categorise colour and pattern for frequency analysis.

Morphological measurements were studied using Principle component analysis (PCA) using Excel XLSTAT 2009 (An add-on that can be brought online). Using the PCA output, assessments of the population data were conducted. For the rest of the study only Snout-Vent length was used as this showed the most variation among populations, it is the measurement least prone to experimental error, and is the most commonly used measurement of reptiles. ANOVA and T-test were conducted using XLSTAT 2009.

Genetic techniques

Tissue for DNA extraction was collected as tail tips. The last 2-3 mm of the tail was removed using clean scissors and stored in 95% ethanol, with the procedure approved by the Massey Animal ethics committee. DNA was extracted using a Qiagen DNeasy tissue and blood kit. DNA was diluted to 1ng/µl for PCR reactions.

Primer pairs, designed to amplify DNA from both mitochondrial and nuclear loci for other reptiles were tested on *Hoplodactylus maculatus* samples (See Appendix 2; list of all primers tested). One mitochondrial gene (16S) and three nuclear loci, (Rag-1, Rag-2 and C-mos) were selected for this study. Primers used were; 16Sd CTCCGGTCTGAACTCAGATCACGTAG, 16Sc GT[A/C]GGCCTAAAAGCAGCCAC (Reeder 1995) C-Mos: Mos-F CTCTGGKGGCTTTGGKKCTGTSTACAAGG, MOS-R GGTGATGGCAAANGAGTAGATGTCTGC (Godinho et al 2006), Rag-1: L2408 TGCACTGTGACATTGGCAA, H2928 GACTGCYTGGCATTCATTTT (Vidal and Hedges 2004), Rag-2: PY1-F CCCTGAGTTTGGATGCTGTACTT, PY1-R AACTGCCTRTTGTCCCCTGGTAT (Gamble et al 2008).

All PCR reactions were done in a thermocycler using the following protocol; 94°C 2mins, 94°C for 20s, 50°C for 15s, 72°C for 130sec and repeated for 40 cycles then

72°C for a further 5mins. Following amplification, the PCR products were sequenced at the AWC genome sequencing centre, using one of the amplification primers: 16Sc, L2408, PY1-F or MOS-F. The preparation of the PCR products for sequencing was done by adding 1ml of the primer used, 2mls of PCR product and 12mls of water.

Analysis of the DNA sequence used the following software; sequencer 4.7 to check the sequences by eye, SE-AL to align the data, check for stop codons and export as a NEXUS file to be used in McClade 4.0. In McClade the characters were compressed and the redundant taxa and characters removed. This left only the base pairs that differed and one sequence for each distinct allele. The data was used to draw minimum spanning trees of alleles for the nuclear loci. The 16S haplotype network was constructed using Automated Nested Clade Analysis v1.0.This program is freely downloaded from the website http://www.rubic.rdg.ac.uk/~mahesh/software.html and can be used to build a TCS haplotype tree using the nexus file of sequences. Hybrid clines were studied using the genetic analysis of all four genes, Analysis V1.3, www.biology.ed.ac.uk/research/institutes/evolution/software/Mac/Analyse/index.html. Other analyses of genetic diversity were conducted using Arlequin 2.0 freely downloaded from http://lgb.unige.ch/arlequin/software/ and GENEPOP 4.0.10 from http://genepop.curtin.edu.au/.

Results

Morphometrics

143 geckos were caught and measured from five locations on the Wellington south coast (Appendix 1). At each location the random sample of 25-30 animals resulted in a mixture of adult and juvenile geckos, of both sexes. Juveniles were excluded from further morphological analysis, leaving 101 adult geckos.

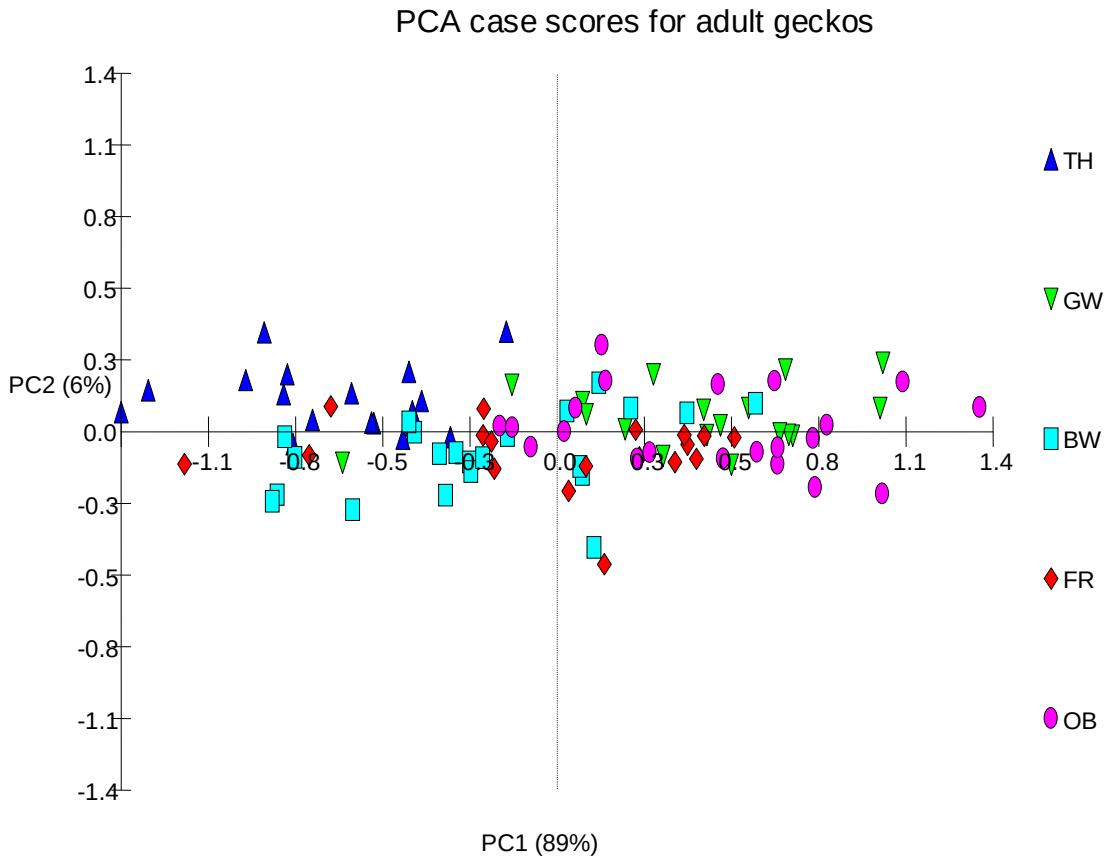


Figure 2 Morphological variation among adult geckos presented as PCA 1 against PCA 2. Individuals in the middle are of intermediate sizes.

Five morphological characters were used in the Principle component analysis; weight, snout-vent length, snout-ear length, head width and head height (Figure 2). Snout-vent length has the highest contribution to PC1 (0.938) followed by weight. The first

Principle component (PC1) accounted for 89% of variation and the second component explained only 6%. When adult males and females are combined PCA fails to clearly differentiate the geckos from each location; however the smallest adult geckos were caught at Turakirae Head and the largest at Ocean Beach (Figure 2). Although a two sample t-test of snout-vent length between males and females in the total sample found no significant difference (P-value > 0.05 at 0.356), male and female geckos were analysed separately due to sexual size dimorphism known to exist at Turakirae Head (Whitaker 1982).

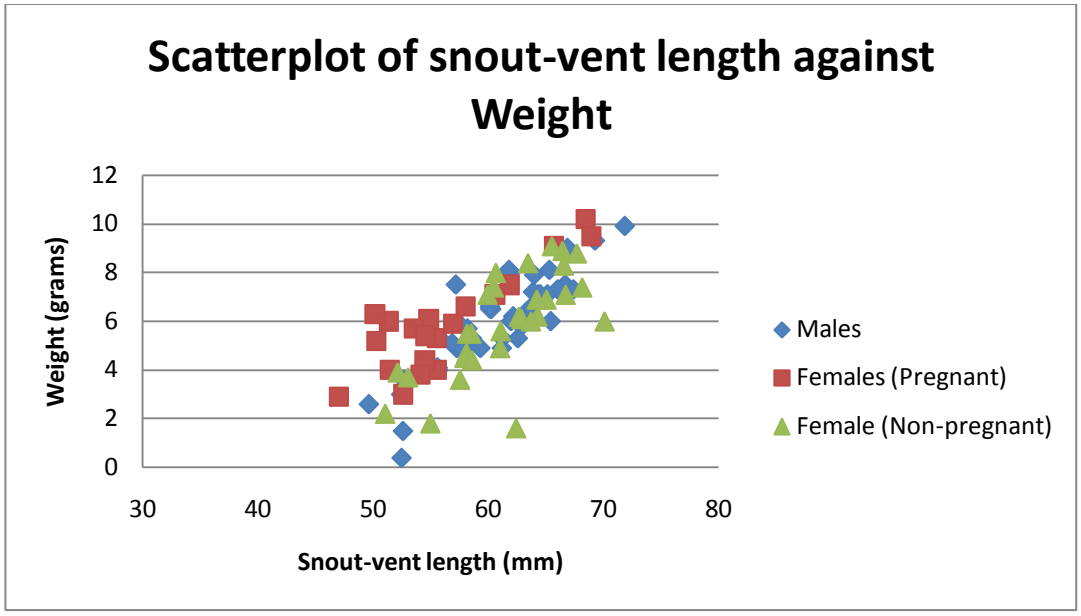


Figure 3 Scatterplot of snout-vent length against weight of males and females. Females were separated into pregnant versus non-pregnant to see if there is any difference between these females.

Even though a number of measurements were initially taken in the field, only a few have been chosen for final assessment of the populations. Weight has been rejected for analysis because of seasonal differences, such as collecting pregnant females during summer and non-pregnant females during winter (Figure 3). Variation in the season the geckos were caught would make analysis of weight an unreliable source of variation.

Head height and head width were also excluded due to difficultly in being consistent in taking this measurement. This main difficultly in taking measurements was making sure the same amount of pressure was applied to each gecko. Although I tried to be careful and accurate sometimes I think I may have tightened the callipers more on some geckos than other making the reading less accurate.

If there is a hybrid zone we expect to see a significant difference between the two end populations on the transit, and intermediate sized individuals somewhere in the middle. The average snout-vent length for adult geckos was compared among populations (Figures 4 and 5). The average snout-vent length between each population increases from one end of the coastal transect to the other. For female geckos this difference on average is about 12mm. This increase in size along the coastal transect will also increase the average weight from approximately 4 grams to 7.5 grams, therefore the overall mass of individual geckos almost doubles from Turakirae Head to Ocean Beach. Fishermans Rock individuals are intermediate in size. Male geckos also show an increase in size from small at Turakirae Heads to large (about 8mm difference), but Gateway not Ocean Beach has on average the largest male geckos (although ANOVA fails to show a significant difference between Ocean Beach and Gateway) (Table 2). The box plots of both male and female average snout-vent length show a steady increase in adult gecko size from one end of the transect to the other.

The Tukey (HSD) test in an ANOVA analysis (Table 1) shows that there is a significant difference in snout-vent length between six of the ten pairwise comparisons for adult females. The four non-significant comparisons of average snout-vent length for females are adjacent locations with the exception of Gateway and Barney's Whare. This pattern of differences is as expected of a cline for morphological size. For the males (Table 2)

however, there is a significant difference in snout-vent length between only four of the ten pairwise comparisons. The populations that differ significantly are Ocean Beach, Gateway, Turakirae Head and Barneys Whare, thus the further the populations are from each other the more different they are.

Table 1 ANOVA results of female snout-vent lengths. It is clear that there is a significant difference between populations further apart.

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
OB vs TH	12.352	7.305	2.834	< 0.0001	Yes
OB vs BW	6.103	3.342	2.834	0.013	Yes
OB vs FR	5.575	3.125	2.834	0.024	Yes
OB vs GW	1.882	0.971	2.834	0.867	No
GW vs TH	10.471	5.785	2.834	< 0.0001	Yes
GW vs BW	4.221	2.179	2.834	0.205	No
GW vs FR	3.694	1.946	2.834	0.308	No
FR vs TH	6.777	4.119	2.834	0.001	Yes
FR vs BW	0.528	0.296	2.834	0.998	No
BW vs TH	6.249	3.696	2.834	0.005	Yes
Tukey's d critical value:			4.008		

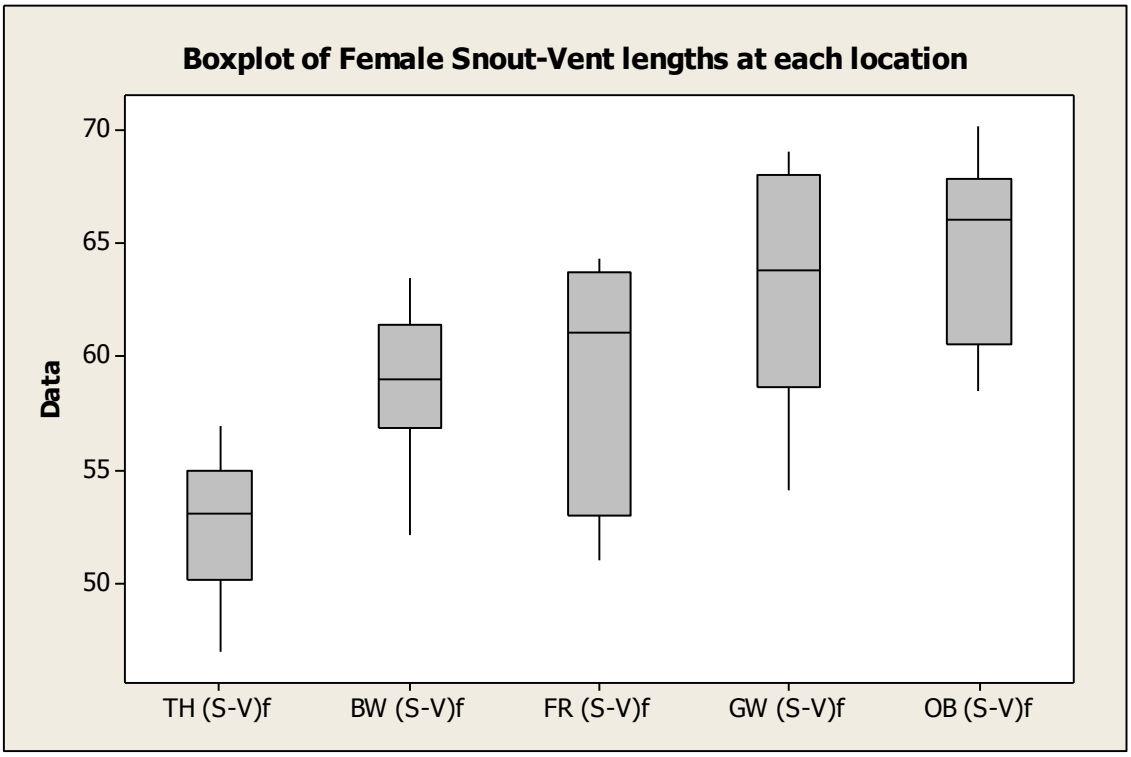


Figure 4, Box plot of snout-vent length of female geckos at each location in the transect showing a cline in size from 53mm to 65mm over 15km

Table 2 ANOVA results for male snout-vent lengths showing significant difference between populations that are further apart.

Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
OB vs TH	8.584	3.486	2.847	0.010	Yes
OB vs BW	6.101	3.532	2.847	0.008	Yes
OB vs FR	3.297	1.740	2.847	0.421	No
OB vs GW	0.673	0.374	2.847	0.996	No
GW vs TH	7.911	3.213	2.847	0.020	Yes
GW vs BW	5.428	3.142	2.847	0.024	Yes
GW vs FR	2.625	1.385	2.847	0.640	No
FR vs TH	5.286	2.086	2.847	0.245	No
FR vs BW	2.804	1.533	2.847	0.547	No
BW vs TH	2.483	1.030	2.847	0.840	No
Tukey's d critical value:			4.026		

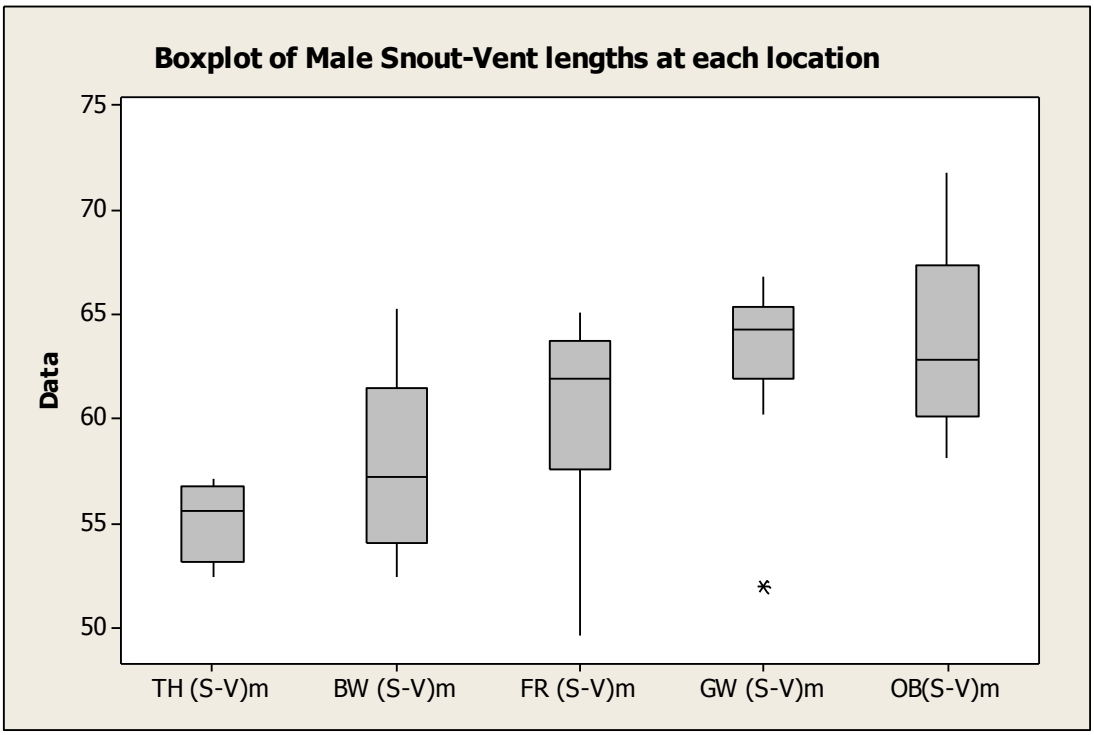


Figure 5 Box plot of snout-vent length of adult male geckos at each location in the transect showing a cline in size from 55mm to 62mm over. The * is an outlier of a small adult gecko in the GW population.

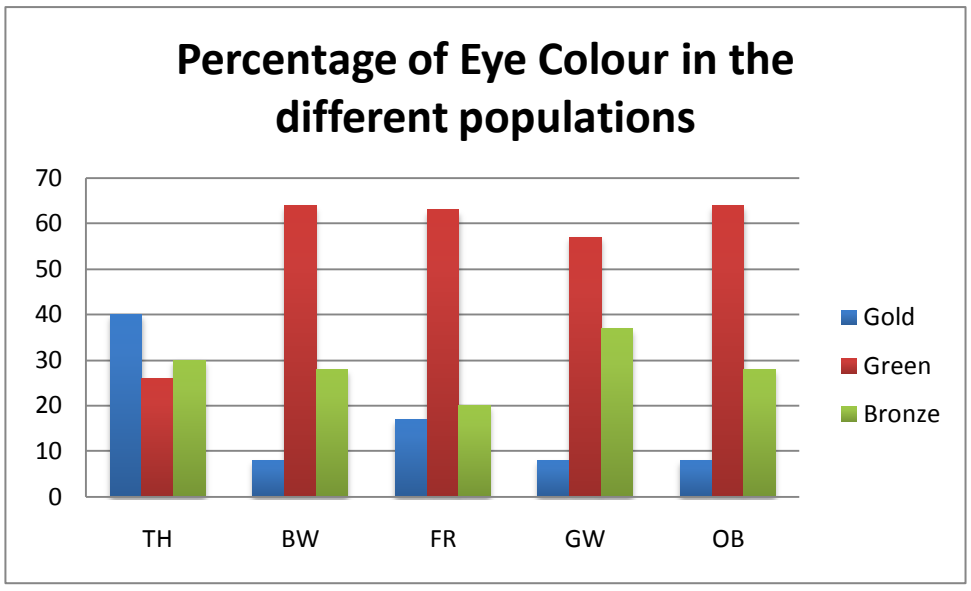


Figure 6 Graph showing the percentage of the three different eye colours in each population. Green eyes are more common in the middle locations and towards Ocean beach but gold is more common at Turakirae Head.

The eye colour of each gecko was scored as one of three colours (green, bronze or gold) and all three colours were recorded in every population (Figure 6). Green eyes were the

most common in four out of five populations. At Turakirae Head gold eyes were more common than anywhere else, and bronze eyes were fairly consistent across the five locations with the fewest at Fishermans Rock.

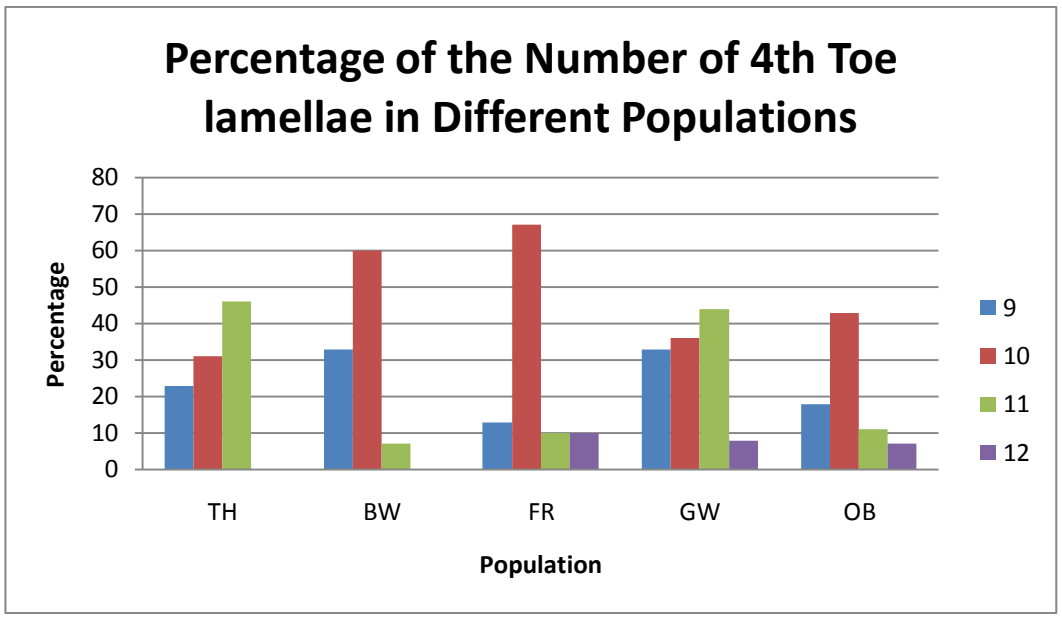


Figure 7 Graph showing the percentage of numbers of 4th toe lamellae in different populations. The averages were the same at around 10 for each location suggesting no significant difference.

As with eye colour, the number of 4th toe lamellae varies among individuals within each population. The range was between 9 and 12 lamellae per toe (Figure 7). The most common number of lamellae was 10 or 11, and the average lamellae number changed only slightly over the zone (9.8-10.5) and did not show a cline. Twelve lamellae per toe were more common from Fisherman's Rock, Gateway and Ocean Beach than the other two populations.

An arbitrary score for dorsal pattern was performed using photos of each gecko to see if populations had a higher frequency of a particular pattern (Table 3). There is no evidence to suggest any significant difference in patterns. However, Turakirae Head geckos had the highest percentage of a striped pattern (38%) than any other location. In all other populations the horizontal bands were the common pattern found, with few

having distinct spots and a few that had patterns that could not be distinguished as either strips, bands, or spots. Colour was not scored because geckos change colour depending on amount of sunlight (Hitchmough per. com.). It was noticed that more geckos caught in winter were darker than geckos caught in summer at the same location.

Table 3 the percentage of geckos with a given score corresponding to pattern. 1 is a pure vivid striped patter and 5 is the pure vivid spotted pattern. The numbers in between are generally in between these two patterns, based on the clarity of pattern and the kind of pattern.

Location	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
TH	38	21	7	28	7
BW	5	11	3	68	11
FR	4	11	22	63	0
GW	19	6	12	44	19
OB	3	40	0	37	10

Genetic analysis

Mitochondrial DNA

Four loci were used to analyse genetic differentiation within and between these gecko populations along the coast and an inland population. Mitochondrial gene 16S was amplified and sequenced from 132 geckos. The length of the fragment in this study was 600 base pairs of which 6.67% (40bp) were variable. From 132 geckos from six locations 23 haplotypes were found. Between 3 and 8 haplotypes were found at each location (Table 4). Nucleotide diversity ranges from a high of 0.014 (Ocean Beach) to

the low of 0.001 (Barney's Whare). Haplotype A is the most common and was found everywhere except Lake Pounui (LP). Lake Pounui (LP) is 12km from Ocean Beach, being more inland and thus is expected to be more distinct from other locations. Each location has one or more unique haplotypes (Table 5). Using GENEPOP to detect population differentiation using the exact G-test for 16S, the only non-significant difference is between Gateway and Fisherman Rock ($P>0.05$). All other population pairs were significantly different from each other (P value ranging from 0.00 – 0.043). This is what we would expect considering the private alleles found at each location and the distribution of the alleles shared between adjacent populations.

A network of the 16S haplotypes shows that ten haplotypes are just one or two mutational steps away from the common haplotype A, which are four steps away from the next haplotype group (Figure 8). Using the haplotype network, and their distribution, the 23 haplotypes were coded into two clusters, split between haplotype G and S (Figure 8). Haplotype A and the ten haplotypes within one or two mutational steps from A are common in Turakirae Head, Barneys Whare and Fishermans Rock (referred to as the south-west group). The other group of haplotypes is more common in Gateway, Ocean beach and Lake Pounui geckos (North-east group). Although these two main haplotype groups change in frequency many populations have both haplotype group (Table 5). This coding of south-west versus north-east 16S haplotypes is used later in the clinal analysis.

Table 4 Genetic diversity found in geckos between Wellington and Wairarapa south coast using mitochondrial DNA sequences (600bp 16S) population statistics, n is the sample size.

Population	n	Number of Haplotypes	Gene Diversity	Mean Number of Pairwise Difference	Nucleotide Diversity
TH	26	7	0.7600	2.270769	0.003797
BW	28	3	0.3624	0.375661	0.000628
FR	21	5	0.7762	5.200000	0.008696
GW	23	8	0.8103	5.454545	0.009166
OB	26	8	0.8667	8.656667	0.014428
LP	8	6	0.9286	5.785714	0.005879

Table 5 the number of individuals with a particular mtDNA haplotype (16S) at each location. Haplotype A is the most common, whereas all others are only present in a few individuals at a few locations. Haplotypes are coded into two groups; red are predominantly south-west and blue is north-east (see Fig 8).

Haplotype	TH	BW	FR	GW	OB	LP
A	12	22	3	2	3	
G	3	1		3		
F		5				
H	4					
N	2					
M	2					
L	2					
R	1					
S			1			
E			4	1		
C			5	7	4	
B			8	7	7	
D				1	4	
T				1		
V				1		
U					1	
W					1	
J					2	2
I					4	1
K						2
O						1
P						1
Q						1

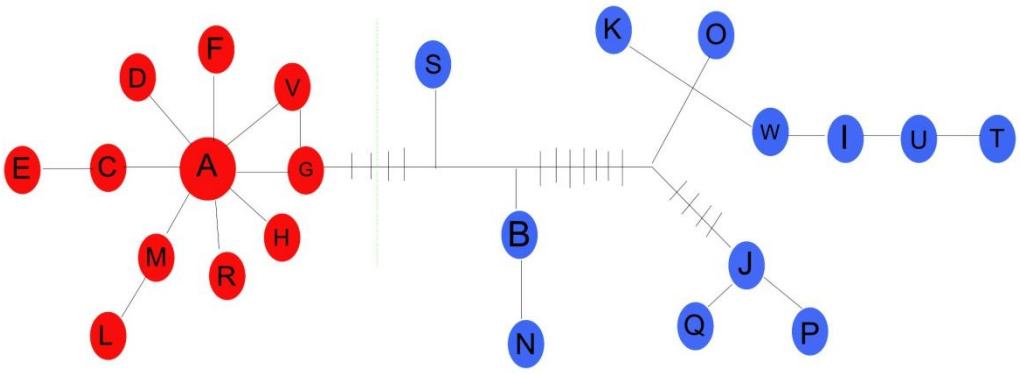


Figure 8 Minimum network tree of mitochondrial haplotypes (based on 600bp of 16S) found in geckos between the Wellington and Wairarapa south coast along a transect. Colours indicate the two codes for the cline analysis: Red equal the small Wellington and blue are the large Wairarapa geckos.

Nuclear DNA analysis

Three nuclear loci were amplified and sequenced for 132 geckos. Genotypes were scored for each gecko using homozygote individuals to identify alleles and heterozygotes inferred from sequences with double peaks at variable nucleotide sites. The Rag-1 product was 327bp long of which 2.44% were variable, with 9 alleles. Rag-2 fragment was 268bp long, 2.24% variable and this locus has 7 alleles. C-mos fragment was 294bp long and had 2.28% variability, and 9 alleles (Table 6). Deviations from Hardy-Weinberg equilibrium were tested for each locus at each location (Table 6). All three loci were in Hardy-Weinberg equilibrium in every population. Gateway population had both the highest frequency of heterozygotes (78%, Rag-1) and the lowest (9% at c-mos) (Table 12). Heterozygosity of adults was compared to that of the juveniles and found to be higher in adults than juveniles. Pairwise linkage-disequilibrium was tested for each population and no evidence was found that the loci are linked, with the exception of Rag-1 linked to C-mos in Turakirae Heads and C-mos linked to Rag-1 and Rag-2 at Fishermans Rock. However, with Bonferroni correction

for multiple test this is not significant (P>0.005). Linkage disequilibrium between mitochondria and nuclear loci is also non-significant (P>0.05) (tested in GENEPOP 4.0.10).

F-Statistics using Arlequin 2.0 gave an average F_{ST} over the three loci of 0.78 (which is significantly greater than 0: P>0.05). The AMOVA results indicated that only 7.76% of total variation is explained by differentiation between populations. However, 92.24% of the variation is within populations.

The tables 7-9 list the number of geckos with a particular allele present in their genotype. The alleles are listed in order of those found in Turakirae Heads to those found in Lake Pounui, showing a cline-like structure to the alleles present in each population.

Rag-1

The most common Rag-1 allele (A) is between one and three mutation steps away from the other eight alleles observed (Figure 9). All locations had one or more alleles found nowhere else, with the exception of Barney's Whare and Ocean Beach (Table 7). Although frequency of alleles varies among sample locations there was not a set of alleles (or a clade) restricted to either end of the zone.

Rag-2

For Rag-2 the most common allele (A) is between one and three mutation steps away from the other 6 alleles (Figure 10). Only Fisherman Rocks has a unique allele found nowhere else, where as all other locations share their alleles with one or more other populations (Table 8). As with Rag-1, the frequency of alleles varies but there is no set of alleles that are restricted to either ends of the zone.

C-mos

The most common allele (A) is only a single mutation step away from 7 of the other alleles, with the exception of allele D which is two mutational steps away from allele A (Figure 11). Gateway, Ocean Beach and Lake Pounui have between one and two unique alleles not shared with other populations (Table 9). As can be seen from the tree there is no clade structure that restricts allele sets to one end of the zone from the other.

Table 6 summary table for the three nuclear loci over coastal transect of geckos, n is the sample size.

		Rag-1	Rag-2	C-Mos	Rag-1	Rag-2	C-Mos
Population	**n**	**Gene Diversity**			**Hardy-Weinberg Equilibrium: P-Value (Number of alleles)**		
TH	26	0.565	0.774	0.111	0.389 (5)	0.216 (5)	1.0 (2)
BW	28	0.642	0.667	0.143	0.033 (4)	0.263 (5)	0.174 (3)
FR	21	0.524	0.703	0.511	0.250 (5)	0.379 (6)	0.110 (3)
GW	22	0.644	0.548	0.086	0.195 (6)	0.809 (5)	1.0 (3)
OB	26	0.540	0.608	0.217	0.049 (3)	0.111 (6)	1.0 (5)
LP	8	0.714	0.241	0.339	0.904 (5)	1.0 (3)	1.0 (3)

Table 7, the number of gecko individuals that have a copy of each allele present in that population for the nuclear gene RAG-1 from Turakirae Head along the coast to Lake Pounui.

Allele	TH	BW	FR	GW	OB	LP
A	32	30	27	20	23	8
B	12	14	11	19	26	3
C	6	8	2	2	2	
E	1	3				3
F	1					
G			1			
D			1	3		
I				1		
H						1

Table 8 the number of alleles present in each population for the nuclear gene RAG-2 from Turakirae Head to Lake Pounui

Allele	TH	BW	FR	GW	OB	LP
A	12	30	15	30	31	14
B	13	6	3	3	1	1
D	7	9	18	4	8	1
E	3	4	2	7	7	
C	17	7	3		4	
H			1			
G				2	1	

Table 9 the number of individuals in each population with a particular allele from the gene C-Mos from Turakirae Head to Lake Pounui

Allele	TH	BW	FR	GW	OB	LP
A	49	51	27	44	46	13
B	3	4	1			
C			13		3	
E				1		
I				1		
D					1	
J					1	
H					1	2
F						1

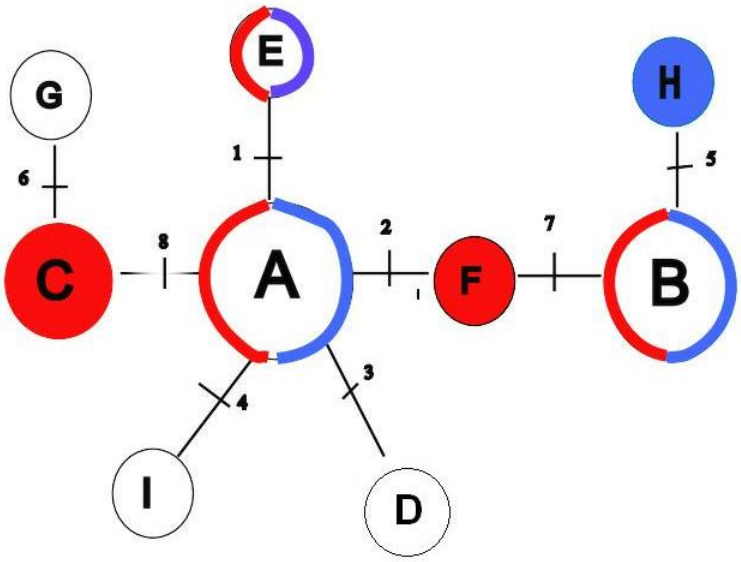


Figure 9, Network of Rag-1 alleles from Wellington and Wairarapa geckos. Red are alleles present in the Turakirae Head population whereas blue are alleles present in the Lake Pounui populations (the two extreme populations of the transect).

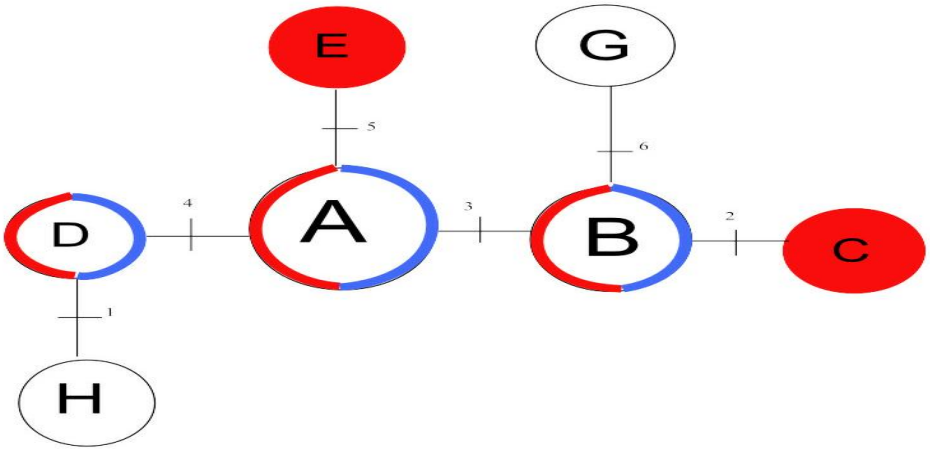


Figure 10 Network of Rag-2 alleles from Wellington and Wairarapa geckos. Red are alleles present in the Turakirae Head population whereas blue are alleles present in the Lake Pounui populations (the two extreme populations of the transect).

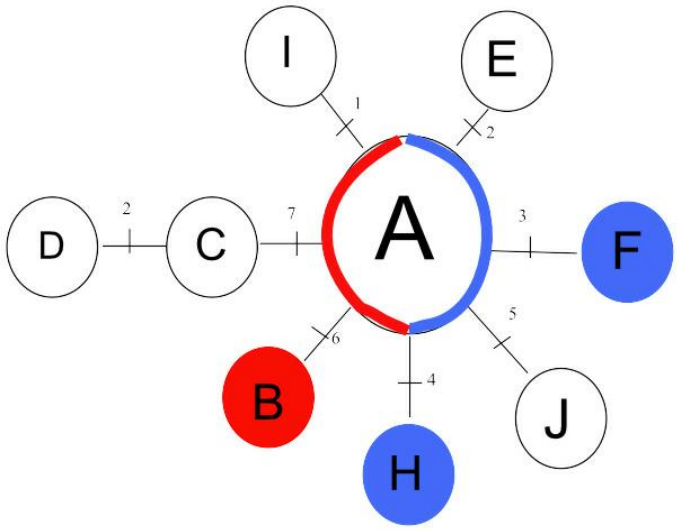


Figure 11 Network of C-Mos alleles from Wellington and Wairarapa geckos. Red are alleles present in the Turakirae Head population whereas blue are alleles present in the Lake Pounui populations (the two extreme populations of the transect).

As with the mitochondrial loci, GENEPOP was used to assess the population differentiation (exact G test) for the three nuclear loci (Table 10). Table 11 shows the F_{ST} values using a Pairwise distance method for all three nuclear loci pooled together. For Rag-1 the significant differences were greater in populations not adjacent to each other. Lake Pounui genotypes were significantly different from all other locations, a result expected considering the distance between Lake Pounui and the other locations. As with Rag-1 the other two loci also showed a trend of a greater difference between populations further apart. However, not all results were as expected, for example Ocean Beach and Lake Pounui were non-significant for Rag-2 and C-mos. Table 10 and 11 work together to show that the further apart the populations are the greater the differences. With correction for multiple tests the significance level drops to P= 0.005, and the number of pairs that are now significant drops.

Table 10 shows the significance level (P = 0.05) for genetic differentiation between each location for the four loci; Rag-1, Rag-2, C-mos and 16S.

Rag-1		Rag-2		C-mos		16S	
Location pair	**P-value**	**Location Pair**	**P-Value**	**Location Pair**	**P-value**	**Location Pair**	**P-value**
TH-BW	0.757	**TH-BW**	0.005	**TH-BW**	1.000	**TH-BW**	0.000
TH-FR	0.524	**TH-FR**	0.001	**TH-FR**	0.000	**TH-FR**	0.000
TH-GW	0.046	**TH-GW**	0.000	**TH-GW**	0.135	**TH-GW**	0.000
TH-OB	0.012	**TH-OB**	0.000	**TH-OB**	0.040	**TH-OB**	0.000
TH-LP	0.017	**TH-LP**	0.000	**TH-LP**	0.015	**TH-LP**	0.000
BW-FR	0.120	**BW-FR**	0.070	**BW-FR**	0.000	**BW-FR**	0.000
BW-GW	0.007	**BW-GW**	0.017	**BW-GW**	0.079	**BW-GW**	0000
BW-OB	0.005	**BW-OB**	0.269	**BW-OB**	0.069	**BW-OB**	0.000
BW-LP	0.025	**BW-LP**	0.090	**BW-LP**	0.013	**BW-LP**	0.000
FR-GW	0.321	**FR-GW**	0.000	**FR-GW**	0.00	**FR-GW**	0.267
FR-OB	0.064	**FR-OB**	0.022	**FR-OB**	0.006	**FR-OB**	0.011
FR-LP	0.037	**FR-LP**	0.006	**FR-LP**	0.005	**FR-LP**	0.000
GW-OB	0.258	**GW-OB**	0.188	**GW-OB**	0.222	**GW-OB**	0.043
GW-LP	0.032	**GW-LP**	0.243	**GW-LP**	0.044	**GW-LP**	0.000
OB-LP	0.001	**OB-LP**	0.129	**OB-LP**	0.186	**OB-LP**	0.004

Table 11 Distance Method; Pairwise differences, Pooled F_{ST} data for three nuclear loci under diagonal matrix and p-values above diagonal.

	TH	**BW**	**FR**	**GW**	**OB**	**LP**
TH	**0**	0.0175	0	0	0	0
BW	0.035	**0**	0.0003	0.0149	0.0089	0.0542
FR	0.099	0.07	**0**	0	0.0003	0.0009
GW	0.13	0.033	0.132	**0**	0.4255	0.0146
OB	0.122	0.035	0.097	-0.004	**0**	0.0148
LP	0.174	0.046	0.13	0.043	0.053	**0**

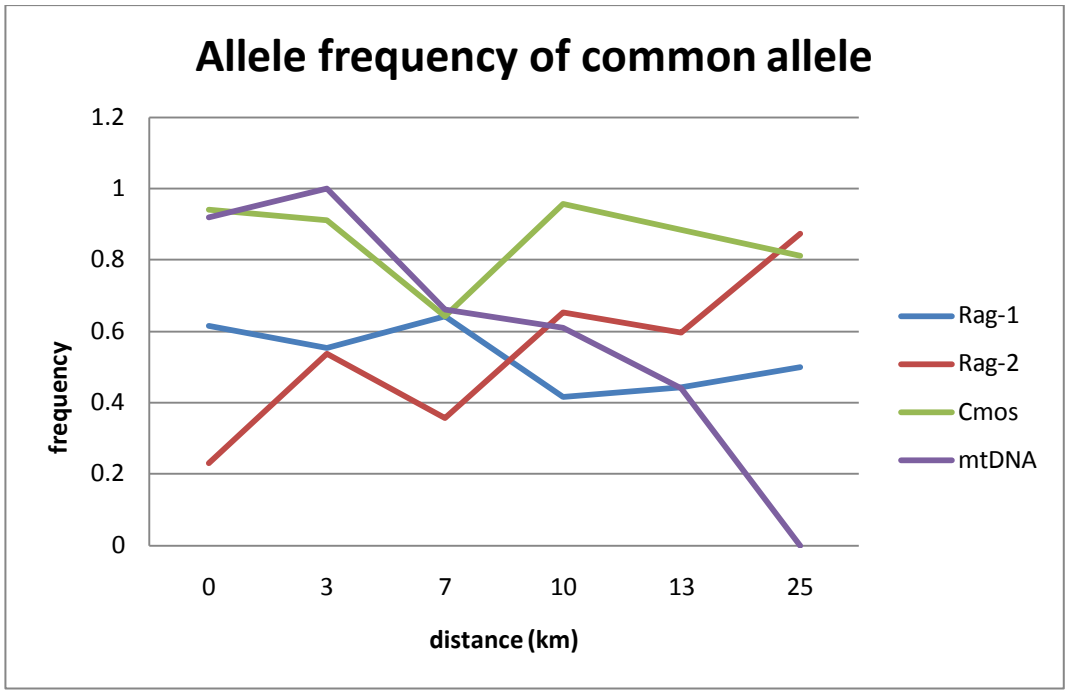


Figure 12, Allele frequency of the most common allele at each of four loci across the coast transect from Wellington to Wairarapa for six gecko populations. Frequency clines are shown by mtDNA and Rag-2.

Table 12 Percentage of heterozygote's (combined adults and juveniles) at each location over the three nuclear loci

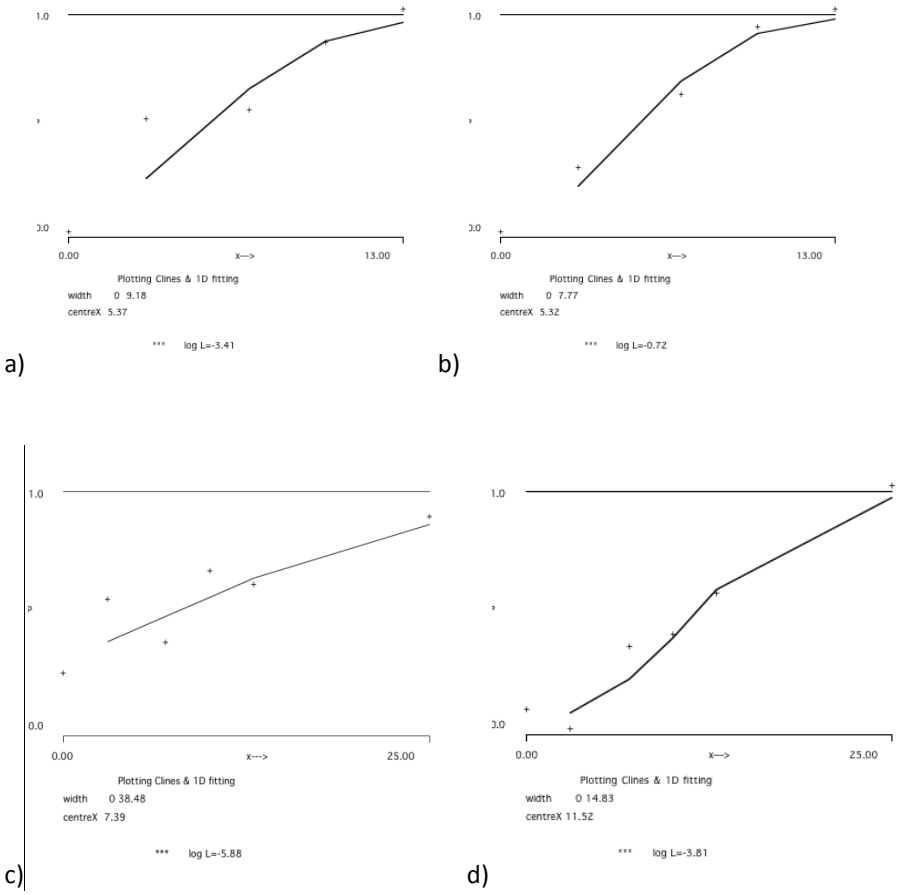
COMBINE	Rag-1	Rag-2	C-Mos
TH	54	62	12
BW	54	64	11
FR	62	62	67
GW	78	61	9
OB	73	62	23
LP	75	25	38

Cline analysis

For the three nuclear loci the change in the frequency of the commonest allele was examined over the transect (Figure 12). Only the common Rag-2 allele shows a clinal change in frequency and was therefore used to examine the hybrid zone. Cline widths and centres were found using Analysis 1.3 (Baird 1996). For morphology, hybrid scores were snout-vent measurements scaled using the equation ((population mean – min. mean)/ (max mean – min. mean)) (Leache & Cole 2007). Table 13 shows the estimates of centres and widths for three characters: mitochondrial DNA, Rag-2 nuclear DNA and snout-vent lengths for males and females separately. Although the estimates of cline centres and widths are not identical they all overlap broadly, as expected of a hybrid zone. The widest cline estimate is for Rag-2 and the narrowest is for female snout-vent length (Table 13). The centre of the mitochondrial cline is significantly further north-east than any of the other three clines.

Table 12 Hybrid cline centres and widths for 4 characters.

	Centre	**Width**
Mitochondrial (16S)	11.52 (11.21-11.97)	14.82 (14.83 – 16.07)
Rag-2	7.39 (6.93 – 7.99)	38.56 (34.98 – 42.27)
Male Snout-vent length	5.32 (5.14- 5.58)	7.77 (7.47-8.76)
Female Snout-vent length	5.37 (5.28-5.62)	9.18 (8.63-9.62)



a) b)

c) d)

Figure 13 Clines of the 4 characters that were used in cline analysis. A) is the female snout-vent length, b) the male snout-vent length, c) Rag-2 nuclear cline and d) is the mitochondrial cline.

Discussion

Adult geckos from the north-east end of the transect (Wairarapa) are longer and heavier than adult geckos from the south-west end of the transect (Wellington). Over the 15km sampled, the average adult female gecko size differed by 12mm and male geckos differed by approximately 8mm. The increase in mass approximately doubles for both males and females from Turakirae Head to Ocean Beach. Over the same 15km a change in allele frequency is seen in one nuclear locus and the mitochondrial 16S locus. This is strong evidence for a hybrid zone.

The two ends of the cline are very different from each other based on morphological and genetic variation. The adult size of geckos from Turakirae Head does not overlap with adults from Ocean beach at the other end of the zone. Size of the geckos alone can be used to distinguish between the two populations (Figure 2). As well as the snout-vent length of geckos the overall bulk of the animal changes along this transect, with Ocean beach geckos getting considerably bulkier than Turakirae Head geckos. Although the weights were influenced by both season and tail loss, it is clear from the comparisons of adult males and field observations that there is an increase in mass.

The significant genetic difference between the two end populations, Turakirae Head and Lake Pounui, is seen in the mitochondrial DNA locus. There are no shared haplotypes between these two populations (Table 5, Figure 8) despite the relatively short distance between them of only 25km (this includes the inland Pounui site). Among the three nuclear loci (Rag-1, Rag-2, and C-mos) there are alleles shared between the extreme populations, however, there are also alleles that are found in one population but not the other (Table 7-9; Figure 9-11). Turakirae Head and Ocean beach are very different from each other sharing only one haplotype. In addition, Ocean Beach and Lake Pounui are

different from each other sharing only two haplotypes. The geographic distance between Turakirae Head and Ocean beach is only 15km and between Ocean beach and Lake Pounui is 10 km. These changes occur over short distances. For all four loci the genetic differentiation between Turakirae Head and Lake Pounui is significant (p-value= 0.05) (Table 10). The F_{ST} pairwise differences for the three nuclear loci confirm that there is a significant difference between the two populations, Turakirae Head and Lake Pounui. This is also seen between Turakirae Head and Ocean Beach geckos (Table 11)

Morphological variation is high, with polymorphism of toe lamellae number, eye colour, colour pattern and hue; however, none of these characters or combinations of characters can distinguish isolated populations. Genetic diversity is very high with all sampled populations having unique (private) alleles at some or all loci studied. This level of morphological and genetic diversity suggests both large populations and limited dispersal.

An interesting observation in this study is the amount of genetic variation found for the mitochondria and three nuclear loci. Rato & Harris (2008) also noted high intraspecific variation in their study species of geckos. They noted that compared to their nuclear markers (Rag-1, C-Mos), their mtDNA had more variation, as found in this study with 23 alleles for the mitochondria and between 7-9 alleles for the three nuclear alleles. However, the 7-9 alleles for the nuclear markers used in this study are high compared to many studies of reptile populations. Godinho et al (2006) in their study on the Iberian lizard found only 4 alleles for their C-mos gene and Arnold et al (2008) found six Rag-2 alleles across ten islands and eight islets in the Cape Verde Islands, covering more than 300kms. The variation in this study is as expected for a reptile species. For all loci used, mitochondria has the highest variation at 7.16% and the three nuclear loci < 3%. Note

that 7% is higher than the barcoding definition of species (Herbert et al 2003; Rubinoff et al 2006), which uses a more rapidly evolving gene (COI). Leache and Cole (2007) found in their fence lizard hybrid zone a maximum of 10.63% divergence which is also significantly higher than the DNA barcode cut off.

Hybrid fitness

The width of a hybrid zone can be maintained by two types of selection, either by a balance of selection against hybrid individuals and the dispersal distance of individuals (endogenous) or by an environmental gradient (exogenous) (Endler 1977; Barton & Hewitt 1985; Barton & Gale 1993; Kruuk et al 1999; Fitzpatrick & Shaffer 2004; Gee 2004). I found no evidence of reduced fitness of hybrids compared to the parents. There is a high level of heterozygosity in all populations at each location for two of the three nuclear genes (Rag-1, Rag-2, and Table 12). Fishermans rock population (near the centre of the clines) has for all three loci around 60% heterozygotes made up of adults and juveniles, however for the c-mos marker the other five populations either side of Fishermans Rock have a lower heterozygosity level compared to those same locations for Rag-1 and Rag-2. This may be the result of selection against hybrids at this locus with locus-specific selection acting on the c-mos gene but not the other two genes. However, c-mos did not have alleles specific to either end of the transect, but allele C was more common at Fishermans Rock than anywhere else sampled. A comparison between juveniles and adults show no significant lowering of heterozygosity as would be expected by hybrid disadvantage. In fact, heterozygosity in adults was higher than juveniles, suggesting heterozygotes may have an advantage (heterosis). However, juveniles showed a larger range among populations, from low to high heterozygosity. Heterozygosity at each locus was not significantly different from expected of Hardy-

Weinberg. In addition, there is no evidence of linkage of loci as might be expected if parental genotypes are at a selective advantage.

What may this mean for selection? It is often very difficult to determine which of the two kinds of selection is acting on the zone (Kruuk et al 1999; Johansson et al 2008) because both models produce similar shaped clines (Gifford 2008). If it was endogenous selection we would expect to see strong selection against hybrids and cline shapes that are coincident and concordant for unlinked markers (Marshall and Sites 2001). This kind of selection will form hybrid zones called "tension zone model" (Brumfield et al 2001; Ruegg 2008). We see this concordant and coincident cline shape in our zone but we lack evidence of strong selection against hybrids. The other form of selection, exogenous, is where the populations' ability to adapt to the local environment is selected against (Barton and Hewitt 1985; Kruuk et al 1999; Gee 2004). The conditions for this form of selection involve selection to act independently on each locus (Kruuk et al 1999; Durret et al 2000). We may not see any effect on the genotypes present in the populations because those genotypes do okay in that location. The clines for different traits may also be disconcordant due to the selection pressures on each locus and/or trait (Durret et al 2000) and is often referred to as the "ecotone model" (Endler 1977).

There have been a number of studies of hybrid zones showing each of the different selection types influencing hybrid zones. For example Bert & Arnold (1995) found evidence for both exogenous and endogenous selection in the hard clam *Mercenaria* species located in a polyhaline lagoon in east-central Florida. A narrow hybrid zone between chickadee species (*Poecila atricapilla and P. carolinensis*) appears to be maintained by endogenous selection (Bronson et al 2003), Phillips et al (2004) found endogenous selection acting on the hybrid zone of *Carlia rubrigularis* skinks. All

studies confirmed the endogenous selection based on the hybrid fitness. An example of exogenous selection is in a quail hybrid zone (*Callipepla californica and C. gambelii*) where the hybrid zone crosses an environmental gradient of precipitation and vegetation (Gee 2004). More examples consider exogenous as a contributing factor but not the dominating force for maintaining a hybrid zone (Johansson et al 2008).

Further study looking at the populations in more depth to gather more information may better show the selection method acting on the hybrid zone and the populations.

Average F_{ST} from the three nuclear loci (0.78) indicates that there is considerable differentiation among the populations. This is what we would expect considering the movement of geckos throughout their life time based on mark-recapture studies. For example, in a study of *H. maculatus* in the South Island 90% of animals recaptured were less than 10m away from their original capture site (Lettink 2007). So migration is limited and gene flow would be low. As in many other studies of population structuring, population differentiation is relatively high, such as in the Iberian lizards with a F_{ST} = 0.46 found throughout their whole distribution of the species in the Iberian peninsula (Godinho et al 2006), Genets (type of cat) from South Africa had significant F_{ST}, but in contrast their South African distribution is much larger (but data not shown) (Gaubert et al 2003).

The morphological data suggest that the hybrid zone is around the Fishermans Rock area where individuals are of intermediate size. Estimates of the cline centres for the genetic markers are coincidence, but only mtDNA cline is concordant (same width) as the morphology. Rag-2 has a significantly wider cline than the morphology and mtDNA. However, for the nuclear marker and the male and female snout-vent length the centres lie somewhere around the Fishermans rock location, 5km – 8km from

Turakirae Heads. The centre of the mitochondrial DNA is more to the North-East end closer to Ocean Beach site, but confidence intervals overlap with male snout-vent length. A shift in mtDNA cline has been observed in many studies including Hispaniolan lizard (Gifford 2008); Swainsons Thrush (26km difference between mtDNA and other markers) (Ruegg 2008) and in Fence lizards (7km difference between mtDNA and other markers) (Leache & Cole 2007). The reason for this may include that mtDNA is haploid (smaller population size) and being maternal it measures only the movement of females, who may move shorter distances than males (Sequeira et al 2004). Rag-2 and the snout-vent length for both males and females have centres close together but the difference in width may indicate weak selection on the different traits. Linkage disequilibrium does not exist between the three nuclear and mitochondrial loci suggesting that selection on any one locus does not affect other loci.

The cline widths of both the male and female snout-vent lengths are relatively narrow compared to the nuclear DNA, this suggests that selection on morphology is greater than this nuclear marker. So it appears that although there is not a complete reproductive isolation of the geckos, selection based on the phenotype may favour smaller geckos in the south-west and larger geckos in the north-east, creating a narrow zone.

Many studies look at selection factors for their hybrid zone, including climate (Ruegg 2008), and habitat/ecotypes (Leache & Cole 2007). There is no major physical or climatic barrier along the coastal transect, so it seems unlikely that the environment is playing a major role in limiting geneflow between these geckos. However, rock type and vegetation do vary from one location to the next. Turakirae Head site is dominated by muehlenbeckia shrubs and rocks and Ocean beach dominated by river beds and

forest of Kanuka (*Kunzea ericoides*) trees and Tutu (*Coriaria arborea*). Ecological measures of habitat were not made so it is not known whether habitat affects the clines positions. If there is a transition in habitat it may have some influence on the size of the geckos creating a narrow hybrid zone for size. Genotypes are shared among the populations supporting the idea that geneflow and hybridisation occurs between adjacent populations. A study on two species of quails showed that geneflow was limited to the region of contact within the zone (Gee 2006) and therefore further sampling away from the zone would be informative.

In many studies of species that form a steep transition along a transect indicate an environment barrier (Gifford 2008). In Gifford's (2008) study on the Hispaniolan lizard the contact zone was on an ecotone characterised by a difference in temperature and precipitation. Gee (2006) had found clines of genetic, plumage and morphometric trait coincides with vegetation and climatic gradients. Leache and Cole (2007) study on fence lizards also meet at an ecotone of woodland and grassland.

The age of this hybrid zone is unknown but based on the amount of mtDNA divergence between the two end populations it may be fairly old. The mitochondrial haplotype clade characteristic of Turakirae Head is considerably different from that at Lake Pounui.

Conclusion

Using morphological and genetic techniques I am able to identify features that distinguish Turakirae Head geckos from Ocean beach geckos, or more in general distinguish coastal geckos from inland geckos. There is a significant difference in the average size of the adult geckos between the two populations at Turakirae Head and Ocean beach. Morphologically the trend is that Turakirae Head geckos are smaller than the Ocean beach geckos, based on an average, there is no overlap in the sizes 15km apart. However, between these two populations geckos are often of intermediate sizes. There is sharing of alleles between populations and some alleles are unique to a population but these are usually rare. Mitochondrial DNA may give more information into where a gecko may be from than nuclear DNA but this should not be used alone to distinguish populations, as there is still some minor overlapping variation.

From the large amount of alleles that are shared between populations we can justify that there is a reasonable amount of hybridising between populations. There is enough of a difference between Turakirae Head and Ocean beach to suggest that gene flow is relatively slow. The movement of geckos is known to be limited in other locations and populations large and therefore we have a high differentiation between populations (Lettink 2007).

The width of the nuclear cline (Rag-2) is wide compared to the narrow cline for the morphological characters. This suggests that there is greater selection for size of geckos than for genotype. It is very likely that size is important in how well a gecko does in a particular habitat and that this hybrid zone is evidence of environment-gradient

selection. Characterising the habitat change over the transect is an important step in future work to provide a better understanding of selection factors that may be occurring in the zone.

Due to the amount of hybridising, sharing of alleles and widths of genetic markers it indicated that this zone is not an effective barrier to gene flow and unlikely to facilitate speciation. These populations are still overall similar to each other and therefore cannot be considered different species. Using the biological species concept there is no reproductive isolation between the two populations and that it would be wise to consider the populations as variants or subspecies. No evidence was found of assortative mating (mate choice based on size) to maintain the zone. But it is possible that selection pressures might result in increased frequency should such behaviour arise, and this could lead to the separate populations further differentiating into different species (Schluter 2009). The current divergence between the two end populations and the formation of a narrow hybrid zone with intermediate morphology for size is evidence that selection will form different species if the gene flow and habitat differences remain as they are now.

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Appendix 1 Data table with morphology and genetic data for each individual sampled from each location.

Gecko	Age	Sex	Eye colour	Weight	Snout-Vent	Snout-Ear	Head Width	Head Height	No. Of 4th toe lamellae	Rag-1	Rag-2	cMos	16S
TH001	A	M	Gold	4	55.65	14.27	13.09	4.83		AB	AC	AA	A
TH002	A	M	Gold	3	52.42	13.16	11.42	5.04	11	AA	BB	AA	H
TH003	J	-	Bronze	2	40.37	12.46	9.81	4.38		AC	BC	AA	N
TH004	J	-	Bronze	0.5	32.07	10.15	8.86	4.41		AA	BD	AA	A
TH005	J	-	Bronze	0.5	35.85	8.49	8.29	3.98		AA	AC	AA	A
TH006	A	F	Gold	3.4	47.5	13.4	11.19	5.48	10	AB	AA	AA	A
TH007	A	F	Green	4	55.5	16.67	13.01	5.82	11	AB	CC	AA	R
TH008	J	-	Green	2	45.65	10.69	9.23	3.15	11	AC	AC	AA	H
TH009	A	F	Green	4	51.47	13.6	12.67	6.13	11	AC	DD	AB	G
TH010	A	F	Bronze	3	52.61	15.13	12.06	5.53	11	AB	BC	AA	A
TH011	J	-	Bronze	0.5	27.77	6.51	6.52	2.82	10	AA	AD	AA	G
TH012	A	F	Bronze	5.3	55.52	12.96	12.84	5.1	11	AA	CC	AA	A
TH013	A	F	Green	4.4	54.51	13.57	12.07	5.21	11	AA	BC	AA	A
TH014	J	-	Gold	2.9	42.39	11.75	9.63	5.92	11	AA	CC	AA	M
TH015	A	F	Gold	6.1	54.83	13.94	13.23	6.51	10	BB	BB	AA	A
TH016	A	F	Gold	6	51.38	11.53	12.26	6.54	10	AA	AC	AA	H
TH017	J	-	Bronze	4	48.49	10.7	10.69	5.19	9	AA	AB	AA	L
TH018	J	-	Gold	2.4	40.69	10.19	9.82	4.12	11	AB	BC	AA	A
TH019	A	F	Green	5.7	53.51	12.95	12.05	5.26	11	AB	BE	AA	N
TH020	A	F	Green	5.2	50.26	12.46	11.81	5.47	9	BC	CC	AB	L
TH021	J	-	Gold	1.8	41.03	8.78	7.96	4.65	10	-	-	-	-
TH022	A	F	Gold	5.9	56.97	12.47	11.01	4.98	11	-	-	-	-
TH023	A	F	Gold	5.4	54.53	11.32	12.17	4.88	9	-	-	-	-
TH024	A	M	Gold	4.1	55.57	12.68	12.74	5.48	9	AA	DD	AA	H
TH025	J	-	Gold	1.8	40.04	8.76	9.33	3.71	10	-	-	-	-
TH026	A	F	Bronze	2.9	47.04	13.15	10.2	4.44	9	-	-	-	-
TH027	A	M	Green	7.5	57.17	15.96	14.83	5.54	10	AC	AA	AA	M
TH028	A	F	Bronze	6.3	50.15	13.85	12.8	5.86	9	CC	AB	AB	A
TH029	J	-	Green	4.7	46.63	11.89	9.67	4.48	11	-	-	-	-
TH030	J	-	Gold	0.9	38.62	11.21	9.78	4.93	10	-	-	-	-

THRAN	-	-	-	-	-	-	-	-	-	AA	BE	AA	A
TH100	-	-	-	-	-	-	-	-	-	BE	AE	AA	A
TH200	-	-	-	-	-	-	-	-	-	BC	CD	AA	G
BW101	A	F	Bronze	7.4	60.49	14.49	14.02	6.7	10	AA	AA	AA	A
BW102	J	-	Gold	3.5	50.41	11.73	10.75	5.65	10	BB	AE	AA	A
BW103	J	-	Green	1.7	37.85	10.37	8.29	3.99	10	AA	BC	AB	F
BW104	A	M	Bronze	4.9	57.25	12.84	11.09	6.19	11	AC	AD	AA	F
BW105	J	-	Bronze	2	42.93	11.31	9.16	4.69	10	AA	AC	AC	A
BW106	A	M	Green	8.1	65.29	15.13	14.29	6.84	9	AA	AC	AB	G
BW107	A	M	Green	8.1	61.78	13.64	12.25	6.44	9	AC	AC	AA	A
BW108	J	-	Bronze	1.4	35.42	9.25	8.34	3.52	9	BE	AD	AA	A
BW109	A	M	Green	4.8	57.79	12.74	11.09	5.68	10	AB	AE	AA	A
BW110	A	M	Green	5.3	55.77	12.4	11.18	6.79	10	AB	AA	AA	A
BW111	A	M	Bronze	5.2	55.54	13.32	11.82	5.01	10	AA	AA	AA	A
BW112	A	M	Green	5.3	58.56	13.83	12.41	5.25	10	-	-	-	-
BW113	A	F	Gold	3.9	52.12	13.35	10.18	4.38	10	AB	CD	AA	A
BW114	A	M	Green	3.6	52.67	11.64	10.41	5.51	9	AC	AD	AA	A
BW115	J	-	Gold	2.3	48.6	12.46	11.63	5.54	10	AA	EE	AA	A
BW116	J	-	Green	2.3	50.15	12.04	9.81	5.01	9	AA	AA	AA	A
BW117	A	F	Green	7.1	59.95	13.91	12.21	6.06	9	AC	CC	AA	A
BW118	A	F	Green	4.5	57.93	12.57	10.92	6.09	10	AA	AB	AA	A
BW119	J	-	Bronze	2.1	47.79	11.36	9.88	5.09	10	AC	AA	AA	A
BW120	A	M	Green	1.5	52.58	12.84	9.96	5.25	10	AE	AD	AA	F
BW121	A	M	Green	0.4	52.46	13.25	10.78	6.05	10	-	-	-	-
BW122	A	F	Gold	3.6	57.55	12.07	10.79	5.02	10	BB	AB	AA	A
BW123	A	F	Green	5.6	61.07	12.24	12.23	6.02	9	AC	AB	AA	A
BW124	A	M	Green	5.1	56.86	12.09	11.19	5.02	10	AA	AA	AA	A
BW125	A	F	Green	4.7	58.1	13.56	11.31	5.48	10	AB	AD	AA	A
BW126	A	M	Green	5.3	61.21	13.52	11.19	5.83	9	-	-	-	-
BW127	A	F	Gold	1.8	54.97	12.51	10.22	5.5	10	AB	AD	AA	A
BW128	A	F	Green	1.6	62.42	13.87	12.1	5.99	11	BB	AA	AA	F
BW129	A	F	Green	8.4	63.45	14.45	11.97	6.49	9	-	-	-	-
BW130	A	M	Green	6.2	62.15	15.18	12.92	6.2	10	-	-	-	-
BW7091	-	-	-	-	-	-	-	-	-	BB	BB	AA	A
BW7092	-	-	-	-	-	-	-	-	-	AC	AD	BB	A

BW7093	-	-	-	-	-	-	-	-	-	CE	AD	AA	F
FR150	A	M	bronze	2.6	49.62	11.13	9.92	3.99	10	AB	AB	AC	A
FR151	A	F	bronze	2.2	51.04	12.27	10.83	5.4	10	AD	BD	BC	E
FR152	A	M	green	7.1	65.12	14.1	13.67	7.05	11	AB	BE	AC	A
FR153	J	-	bronze	0.8	32.65	8.35	6.95	3.56	10	AA	AD	AC	C
FR154	J	-	bronze	0.8	31.46	8.25	6.98	3.96	9	-	-	-	-
FR155	A	M	bronze	4.9	61.17	14.07	12.1	6.27	10	BB	DD	AA	B
FR156	A	F	green	6.2	64.29	14.93	12.26	6.2	10	AB	AD	AA	A
FR157	A	F	bronze	3.7	53.03	11.94	10.35	6	12	CG	AA	AA	E
FR158	A	M	green	5.9	57.45	14.18	12.17	6.74	11	AA	DD	AC	C
FR159	J	-	green	2.2	45.23	11.47	9.88	5.03	10	AA	CE	AC	B
FR160	A	M	green	6.1	63.84	14.67	13.42	6.73	10	AA	AA	AA	B
FR161	A	F	gold	6.9	64.21	14.5	13.12	7.02	10	AA	DD	AC	B
FR162	A	M	green	6.4	63.66	14.75	13.7	6.02	12	AA	DD	AC	C
FR163	A	F	green	5	53.03	13.14	11.43	5.9	10	AB	AD	AC	C
FR164	A	F	bronze	6	63.71	14.55	12.14	6.26	10	AB	CD	AC	B
FR165	J	-	green	1.7	41.24	10.39	8.55	4.32	10	-	-	-	-
FR166	J	-	bronze	0.7	28.9	8.23	6.85	2.97	10	AB	AD	AC	B
FR167	J	-	green	0.8	32.35	8.04	7.27	4.02	9	AB	AH	AA	B
FR168	A	F	green	5.5	58.16	13.12	11.62	5.79	10	AB	AC	AC	C
FR169	A	F	green	4.4	58.6	13.29	11.54	5.25	10	AB	AD	AA	B
FR170	A	M	green	4.9	57.81	14.13	12.19	5.75	10	AA	DD	AC	E
FR171	J	-	bronze	2.4	45.06	11.09	9.23	4.53	10	-	-	-	-
FR172	A	F	green	6.2	62.67	14.08	11.91	5.44	10	-	-	-	-
FR173	J	-	bronze	1.7	40.81	10.4	8.79	4.84	9	-	-	-	-
FR174	A	F	green	6.1	62.89	14.89	12.91	6.09	12	-	-	-	-
FR175	J	-	green	3.4	52.22	12.09	11.01	5.56	9	-	-	-	-
FR176	J	-	bronze	1.3	37.58	8.32	7.03	3.98	10	-	-	-	-
FR177	A	M	gold	6	61.96	14.78	13.85	6.57	10	-	-	-	-
FR178	A	F	green	4.9	61.03	12.64	10.94	6.05	10	-	-	-	-
FR179	A	M	green	6.6	63.77	13.29	12.49	6.65	11	AA	AD	AA	E
GW070	A	F	Bronze	6.6	58.04	14.94	13.3	5.16	10	AA	AA	AA	A
GW071	A	F	Dark green	9.1	65.69	17.22	14.14	6.84	11	AB	AE	AA	C
GW072	J	-	Bronze	2.7	43.26	10.42	8.85	4.73	11	AB	AB	AI	A
GW073	A	F	Bronze	8.9	66.45	14.27	11.93	5.99	11	BB	AD	AA	B

GW074	J	-	Bronze	1.2	39.74	10.23	8.29	4.55	11	AB	DE	AA	G
GW075	A	M	green	7.6	65.13	15.9	14.02	6.83	12	BC	AB	AA	B
GW076	A	F	green	10.2	68.47	16.59	15.47	7	10	AC	AA	AA	G
GW077	A	F	green	7.1	60.59	13.92	12.51	5.61	12	BB	AA	AA	B
GW078	J	-	green	1.5	35.62	9.04	8.31	3.51	11	AB	AD	AA	B
GW079	A	M	green	8	61.94	16	13.67	6.63	10	BI	AA	AE	B
GW080	A	M	green	9	66.86	13.99	12.06	6.15	10	AB	AE	AA	G
GW081	J	-	Green	3.3	48.93	11.66	9.97	5.49	10	AB	AA	AA	D
GW082	A	F	Green	7.5	61.88	14.09	11.27	5.98	11	AA	AD	AA	B
GW083	A	M	Green	7.1	64.32	14.59	13.05	6.11	9	AB	AE	AA	C
GW084	A	M	Gold	6.5	60.24	15.31	13.12	6.41	10	AB	AA	AA	C
GW085	A	M	Green	7.5	66.66	15.36	13.53	6.63	9	AB	AE	AA	C
GW086	A	M	Gold	3.9	51.98	12.79	10.51	4.65	11	AB	AB	AA	C
GW087	J	-	Bronze	3.2	52.4	11.81	11.05	4.64	10	AB	AA	AA	C
GW088	A	F	Bronze	3.8	54.11	10.96	11.06	6.39	11	AB	AA	AA	T
GW089	A	M	green	7.2	63.87	15.3	14.1	6.57	10	AD	AA	AA	E
GW090	J	-	Bronze	2.1	45.73	11.15	9.17	5.07	9	AA	AE	AA	B
GW091	A	M	green	6	65.41	14.7	13.23	6	11	-	-	-	-
GW092	A	M	green	6.4	63.34	13.27	12.69	6.13	10	-	-	-	-
GW094	A	F	green	9.5	68.98	15.05	14.23	7.34	11	-	-	-	-
GW095	A	M	green	7.1	64.5	14.84	13.44	7.9	11	-	-	-	-
GWRAN	-	-	-	-	-	-	-	-	-	BD	EG	AA	V
GWRAN2	-	-	-	-	-	-	-	-	-	DF	AG	AA	C
OB033	J	-	Green	5.3	50.85	13.89	12.44	5.46	11	AB	AA	AA	D
OB034	J	-	Gold	5.4	57.95	14.2	13.49	5.93	11	BB	AD	AC	A
OB035	A	M	Green	7.9	63.97	15.7	14.87	6.53	12	AB	AA	AA	B
OB036	A	F	Green	9.1	65.53	16.72	13.32	7.04	12	BB	AD	AA	A
OB037	A	F	Green	8	60.66	14.17	13.35	7.16	10	BB	CG	AA	B
OB038	A	F	Bronze	8.5	60.05	15.69	13.93	7.23	11	AB	AC	AH	B
OB039	A	F	Bronze	7.1	66.72	13.64	12.85	7.37	10	BB	AD	AA	C
OB040	A	M	Green	7.3	67.38	15.28	14.05	7.36	11	AB	AE	AA	A
OB041	A	F	Gold	6	70.11	16.39	13.08	6.25	10	-	-	-	-
OB042	A	M	Bronze	5.3	62.55	14.36	12.81	6.75	10	-	-	-	-
OB043	J	-	Bronze	8	42.21	9.94	8.76	4.1	10	AA	EE	AC	J
0B044	A	F	Bronze	6.9	65.06	13.31	12.61	7.47	10	AB	AA	AA	B

OB045	J	-	Bronze	2	42.62	10.54	9.07	5.86	11	AB	AA	AD	I
OB046	J	-	Bronze	1.1	32.62	9.04	7.72	4.33	10	AB	AA	AA	D
OB047	A	M	Gold	5.8	62.88	14.15	13.03	6.16	10	AB	AE	AA	U
OB048	A	M	Bronze	6.6	60.13	14.65	12.82	6.52	10	AB	AA	AC	W
OB049	A	F	Bronze	5.5	58.53	14.76	11.79	6.13	9	AB	BE	AA	B
OB050	A	F	Green	7.4	68.16	13.63	11.7	6.16	10	AB	AE	AA	J
OB051	A	F	Gold	8.3	66.56	13.49	12.29	6.7	9	BB	AC	AA	B
OB052	J	-	Bronze	0.7	32.42	7.81	6.63	4.11	11	AA	AA	AA	I
OB053	A	M	Gold	4.9	59.3	14.78	11.68	6.41	10	AB	AD	AA	B
OB054	A	M	Bronze	5.7	58.18	13.79	12.02	6.18	9	AB	AD	AJ	C
OB055	A	F	Gold	8.8	67.7	15.57	12.9	5.75	11	-	-	-	-
OB056	A	M	Green	9.9	71.84	16.11	15.02	7.53	9	AB	AA	AA	J
OB057	A	M	Gold	6.5	60.16	13.66	11.91	5.69	11	-	-	-	-
OB058	J	-	Bronze	1.8	43.16	10.33	8.18	3.58	9	-	-	-	-
OB059	A	M	Green	7.3	66.01	13.9	13.06	6.26	11	-	-	-	-
OB060	A	M	Bronze	9.3	69.25	16.92	15.64	7.61	10	-	-	-	-
RAH33	-	-	-	-	-	-	-	-	-	AB	AA	AF	K
RAH34	-	-	-	-	-	-	-	-	-	AH	AB	AA	K
RAH146	-	-	-	-	-	-	-	-	-	DE	AA	AA	O
RAH229	-	-	-	-	-	-	-	-	-	AE	AD	AH	P
RAH230	-	-	-	-	-	-	-	-	-	AB	AA	AA	J
RAH231	-	-	-	-	-	-	-	-	-	AA	AA	AA	Q
RAH232	-	-	-	-	-	-	-	-	-	AA	AA	AA	I
RAH345	-	-	-	-	-	-	-	-	-	AC	AD	AA	C
RAH346	-	-	-	-	-	-	-	-	-	AB	AA	AA	D
RAH347	-	-	-	-	-	-	-	-	-	AB	AD	AA	C
RAH348	-	-	-	-	-	-	-	-	-	AB	AC	AA	I
RAH349	-	-	-	-	-	-	-	-	-	AC	DE	AA	D
RAH355	-	-	-	-	-	-	-	-	-	AC	AA	AH	S
RAH498	-	-	-	-	-	-	-	-	-	BE	AA	AH	J

Appendix 2 Mitochondrial primers and their sequences

MtDNA Gene	**Primer**	**Primer Sequence**	**Source**
Cytochrome b	Cyt b1 (L14841)	AAAAAGCTTCCATCCAACATCTC AGCATGATGA	Kocher *et al*, 1989
	Cyt b2 (H15149)	AAACTGCAGCCCCTCAGAATGAT ATTTGTCCTCA	Kocher *et al*, 1989
With Cyt b2	P1	TGAGGACAAATATCATTYTGRGG	Jesus *et al*, 2007
	MV216	AAATAGGAA$_{G}^{A}$TATCA$_{C}^{T}$TCTGGTT T$_{G}^{A}$AT	Mortiz
	MVZ04	GCAGCCCCTCAGAATGATATTTCC T	Phillips et al 2004
	Ph-1	GACCCCAATACGAAAAACCACCC	Phillips et al 2004
ND2	ND2b	GCCCATACCCCAAAAATGTYG	Jennings et al, 2003
	ND2c	AACCAAACCCAAACACGAAARAT YAT	Jennings et al, 2003
	ND2d	AAACCAAGAGCCTTCAAAG	Jennings et al, 2003
	ND2f	TGTRGTTATRTGDGATATYCG	Jennings et al, 2003
	ND2e	GCGCGCTGGTTTGGGTDWTTAGY	Jennings et

		TGTTAA	al, 2003
	L(mod of Jennings	GCCCATACCCCGAAAATSTTG	Oliver et al, 2007
	H	TTAGGGTRGTTATTTHGAYATKCG	Oliver et al, 2007
	L4645	ACAGAAGCCGCAACAAAATA	Macey et al, 1997a
	L4831	TGACTTCCAGAAGTAATACAAGG	Macey et al, 1997a
	L4882	TGACAAAAACTAGCACC	Macey et al, 1997a
	H4980	ATTTTTCGTAGTTGGGTTTGRTT	Macey et al, 1997a
	L5002	AACCAAACCCAACTACGAAAAAT	Macey et al, 1997a
	H4629	AAGTATTTTGTTGCGGCTTC	Macey et al, 2000
	L4882mod	CAACATGACAAAAATCGCCCC	Pepper et al, 2006
	$tRNA^{Asn}$	CTAAAATRTTRCGGGATCGAGGCC	Read et al, 2001
ND4	ND4 F	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arevalo et al 1994
	ND4 R (LEU)	CATTACTTTTACTTGGATTTGCACCA	Arevalo et al 1994

16S	16SH	CCGGTCTGAACTCAGATCAGGT	
	L2510	CGCCTGTTTATCAAAAACAT	
	H3056	CTCCGGTCTGAACTCAGATCACGTAGG	
	16S-F	CTAACCGTGCAAAGGTAGCGTAATCAC	Gamble et al, 2008
	16Sc	GT[A/C]GGCCTAAAAGCAGCCAC	Reeder, 1995
	16Sb	GCGCTGTTATCCCTAGGGTAACTTG	Reeder, 1995
12s rRNA	12Sa	CTGGGATTAGATACCCCACTA	Kocher et al 1989
	12Sb	TGAGGAGGGTGACGGGCGCT	Kocher et al 1989
	L1091	AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT	Kocher et al 1989
	H1478	TGACTGCAGAGGGTGACGGGCGGTGTGT	Kocher et al 1989
Control region	L15926	TCAAAGCTTACACCAGTCTTGTAAACC	Kocher et al 1989
	L16007	CCCAAAGCTAAAATTCTAA	Kocher et al 1989
	H00651	TAACTGCAGAAGGCTAGGACCAAACCT	Kocher et al 1989

Appendix 3 Nuclear markers and their sequences tested.

Gene	Primers	Primers Sequence	Source
RAG-1	L2408	TGCACTGTGACATTGGCAA	Vidal & Hedges 2004
	H2928	GACTGCYTGGCATTCATTTT	Vidal & Hedges 2004
	H2920	GCCATTCATTTTYCGAA	Vidal & Hedges 2004
	G396	TCTGAATGGAAATTCAAGCTGTT	Groth & Barrowclough 1999
	G397	AAAGGTGGCCGACCGAGGCAGCATC	Groth & Barrowclough 1999
	R18	GATGCTGCCTCGGTCGGCCACCTTT	Groth & Barrowclough 1999
	F700	GGAGACATGGACACAATCCATCCTA	Bauer et al 2007 cited in Gamble et al 2008
	R700	TTTGTACTGAGATGGATCTTTTGCA	Bauer et al 2007 cited in

			Gamble et al 2008
	L-RAG-1b	TTCCAGCCAYTGCATGCTCT	
	H-snRAG-1	ATTGCCAATGTCACAGTGCA	
RAG2	EM1-F	TGGAACAGAGTGATYGACTGCAT	Gamble et al 2008
	EM1-R	ATTTCCCATATCAYTCCCAAACC	Gamble et al 2008
	PY1-F	CCCTGAGTTTGGATGCTGTACTT	Gamble et al 2008
	PY1-R	AACTGCCTRTTGTCCCCTGGTAT	Gamble et al 2008
C-mos	G73	GCGGTAAAGCAGGTGAAGAAA	Saint et al 1998
	G74	TGAGCATCCAAAGTCTCCAATC	Saint et al 1998
	FU-F	TTTGGTTCKGTCTACAAGGCTAC	Gamble et al 2008
	FU-R	AGGGAACATCCAAAGTCTCCAAT	Gamble et al 2008
	Mos-F	CTCTGGKGGCTTTGGKKCTGTSTACAAGG	Godinho et al 2006
	Mos-R	GGTGATGGCAAANGAGTAGATGTCTGC	Godinho et al 2006
	G78	AGRGTGATRGCAAAVGARTAR	

		ATG	
	G155	TGCTACTAWAGCYYTCCAGC	
	G303	AATTATGCCATCMCCTMTTCC	
Phosducin	PHOF2	AGATGAGCATGCAGGAGTATG A	Bauer et al 2007 cited in Gamble et al 2008
	PHOR1	TCCACATCCACAGCAAAAAAC TCCT	Bauer et al 2007 cited in Gamble et al 2008
ACM4	tgF	CAAGCCTGAGAGCAARAAGG	Gamble et al 2008
	tgR	ACYTGACTCCTGGCAATGCT	Gamble et al 2008
	int-F	TTTYCTGAAGAGCCCTCTGGTC	Gamble et al 2008
	int-R	CAAATTTCCTGGCAACATTRGC	Gamble et al 2008
B-fibrinogens	FIB-B17U	GGAGAAAACAGGACAATGACA ATTCAC	Prychitko & Moore 1997
	FIB-B17L	TCCCCAGTAGTATCTGCCATTA GGGTT	Prychitko & Moore 1997
	FIB-B17U2	CATCCATGCAGTTCTGGCAATT CCAAGT	Prychitko & Moore 1997

	FIB-B17L2	TGGGAGGTGAAGCAGCTAAGAAAAACAA	Prychitko & Moore 1997
	FIBL210	ACTTGGAATTGCCAGAACTGCAT	Prychitko & Moore 1997
	FIBU330	CTAGGAACTGCAAGTAA	Prychitko & Moore 1997
Aldolase	Ald 1	CRAAGAAGGATGGAGCTGACTTTGC	Phillips et al 2004
	Ad 2	CRGCCATTCTGTAACACAACAGCCAA	Phillips et al 2004
	Ald-1	TGTGCCCAGTATAAGGATCG	Phillips et al 2004
	Ald-2	CCCATCAGGGAGAATTTCAGGCTCCACAA	Phillips et al 2004
Rhodopsin	Rho3	CRCCTTGCCTGGACACCCTATGCTG	Phillips et al 2004
	Rho4	CRCTCTGGAATAAAGGAGAGGGTCTCT	Phillips et al 2004
Cyt P450	P450 UP1	TTGGAGACGGGCAGTGTGAT	
	P450 UP2	TCCGAGTACGTCCAGGC	
	P450 UP3	CATGTTCGAGGGTCACGACACCACG	
	P450 UP4	ACGCCTATGTACCCTTCAGC	
	P450 UP5	GCCTGGAATAACCTGGACGAC	
	P450 UP6	ACCAAGAAAATGACGCAACAA	

		AG	
	P450 DO1	GCCTGGACGTACTCGGA	
	P450 DO2	GGAGGGTCTTGAAGCAGGCATTA	
	P450 DO3	GTGTAGAGGACCCAGTTGAT	
	P450 DO4	CCAGCGCTGAAGGGTACATAGGCGT	
	P450 DO5	GCGTCGTCGTCCAGGTTATT	
	P450 DO6	ATAAATAGCACCTTCCTCAGTAG	
Myoglobin	Myo2	GCCACCAAGCACAAGATCCC	
	Myo3	CGGAAGAGCTCCAGGGCCTT	
Gapd	GapdH950	CATCAAGTCCACAACACGGTTGCTGTA	
Gapd	GapdL890	ACCTTTAATGCGGGTGCTGGCATTGC	