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# Population Structure in the New Zealand Falcon

*(Falco novaeseelandiae)*



Photo-Lena Olley

A thesis presented in partial fulfilment of the requirements for the degree of Master of  
Science in Conservation Biology

Massey University, Palmerston North

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## Flight

On the slow wing-beat

of a curious falcon

my wishes belonged.

I rose and spiralled

on thermals

and the mountains smiled

all afternoon.

The falcon's wings

splintered the autumn sunlight

above the remnant fog

that sat on the river

like a marquee

and water scrawled inscriptions

on every stone.

-Brian Turner



## **Abstract:**

The New Zealand falcon (*Falco novaeseelandiae*) is a medium sized falcon endemic to New Zealand. New Zealand falcons have a flexible ecology, inhabiting a variety of habitats including bush, coastlines, mountains, open tussock land, farm land and exotic pine forests. Phylogenetic analysis suggests the New Zealand falcon is not sister to or related closely to any species in either Australia or South America as proposed in earlier research. Neither does it appear to fit within in any other major group such as the heirofalcons or the kestrels.

The New Zealand falcon is currently defined as a single variable species with three recognised morphs or races that are referred to as the Bush, Eastern and Southern these appear to differ in colour and size. This proposal was established in 1977 and has since become generally accepted. However, there are alternative hypotheses as to how this variation in size may be spatially partitioned across the New Zealand landscape. A reassessment of the morphometric data in New Zealand falcons is needed to identify how this morphological variance is distributed. Specifically, to identify any evidence for three distinct morphs, in contrast to the alternative hypothesis of a gradient in size consistent with Bergmann's rule. The analysis suggests that there is little support for the occurrence of three morphs of New Zealand falcon; instead, there is a distinct difference in size between the North and South Islands. There is some evidence of a gradual change corresponding to latitude but this appears to be minimal. Mean wing lengths are significantly longer in male and female falcons in the South Island compared to those in the North Island.

To understand if the size difference between the North and South Islands is an effect of an adaptive response and to examine the extent of gene flow occurring between the two islands a study of neutral genetic markers is needed. Evidence of genetic structure was tested for among New Zealand falcon populations using nuclear and mitochondrial data. Little support for any population structuring was identified. Evidence from this analysis suggests that the falcons are responding to particular environmental conditions within each island resulting in a change in size, however high juvenile dispersal may be preventing the partitioning of gene flow between the North and South Islands.



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## **Preface**

This thesis has been written and organised as self-contained chapters that will act as submissions to peer-reviewed scientific journals. Because of this, individual chapters will contain unavoidable repetition. This thesis is original work of the author, unless stated otherwise in the references, methods and acknowledgments.

## **Note on names**

Latin names are given once in each chapter for each species, otherwise common names are used. The exception is in chapter two where latin names are used in the results for ease of translation with figures in which only latin names are used. The discussion of chapter two then gives the latin names once again.



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# Chapter 1

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## Introduction to the New Zealand falcon (*Falco novaeseelandiae*, Gmelin, 1788)



Female and male New Zealand falcon siblings, Marlborough (Photo-Lena Olley)

## 1.1 The New Zealand falcon:

The New Zealand falcon (*Falco novaeseelandiae*) is a medium sized falcon endemic to New Zealand. They have a flexible ecology, inhabiting a variety of habitats including bush, coastlines, mountains, open tussock land, farm land and exotic pine forests. As with most falcon species, the New Zealand falcon is sexually dimorphic with the female being up to a third larger than the male (more detail later in chapter). They are skilled hunters, taking only live prey. Prey items include small and medium sized birds, rodents, rabbits, hares, reptiles and insects, although, in the past their dietary range may have been more restricted. In the Canterbury region, fossil deposits around former falcon nest sites that date to the Polynesian period before 1850 contained a large number of parakeet bones (Worthy & Holdaway, 1995). Recent research has found falcons will take the prey that is most abundant (Seaton, 2007); and in most parts of modern New Zealand small European birds such as sparrows and finches predominate.

The New Zealand falcon is widespread and can be found throughout most of mainland of New Zealand as well as offshore islands including Stewart Island and the Auckland Islands. The species seems to be absent in places such as Northland, and possibly parts of Canterbury (Bell & Lawrence, 2009) however further investigations are needed here as there have been some reported tentative sightings (pers. com.). The falcon was present on the Chatham Islands at one time. William Travers (1866) reported that a large species of falcon was present. Walter Buller (1888) recorded an egg from the Chatham Islands in the Canterbury Museum. Dawson (1957) summarised the numerous records of recent fossil falcon bones from the Chatham Islands, where they were first collected by Henry Forbes in Jan-Feb 1892 (Dawson, 1957). Aikman & Miskelly (2004) infer that in the Chatham Islands New Zealand falcon was locally extinct circa 1890.

The New Zealand falcon is currently defined as a single variable species with three recognised morphs or races that are usually referred to as the Bush, Eastern and

Southern. There is uncertainty surrounding the geographic boundaries of the three morphs but morphological, ecological and behavioural variation in the populations suggests that they are genetically isolated (figure 1.1) (Fox, 1977). Bush and Eastern falcons are currently defined as having ranges that likely meet or overlap around the upper and western South Island, suggesting that they are not spatially isolated. It has been proposed that Southern and Bush falcons were formerly a single type that became separated around the middle of the west coast of the South Island (Fox, 1977).

This model of falcon races (Fox, 1977) has become generally accepted by most New Zealand ornithologists. The Department of Conservation (DOC) includes the three morphs as separate units in the latest threat classification list (Robertson et al, 2013), most New Zealand bird books refer to three morphs (Heather & Robertson 1996, Scofield & Stephenson 2013, Watola 2009), and even the International Union for Conservation of Nature (IUCN) records the three forms (<http://www.iucnredlist.org>). There are tentative plans for separate management of each race, and currently the Department of Conservation does not allow translocations between some of the geographical ranges (pers. obs.).

The population size of the New Zealand falcon is not well known. Fox (1977) suggested that there were between 3000 and 4500 pairs, but they were in decline. Of these, about 3100-3200 pairs were thought to be Eastern, 450-850 Bush, and 140-280 Southern Falcons (Fox, 1978). These estimates are frequently requoted (Bell & Lawrence (2009) and Heather & Robertson (1996).



Figure 1.1: Geographic ranges of the three New Zealand falcon morphs or races (Bush, Eastern and Southern) as proposed by Fox (1977).

Threats to the New Zealand falcon are not well understood, but the most severe, like as with most New Zealand species, is predation by introduced mammals. Falcons are particularly vulnerable in the breeding season due to their tendency to nest on the ground. Recently, it has been discovered that electrocution on power lines may be a significant cause of mortality in falcons (Fox & Wynn, 2010). There are also a number of reports of deliberate killing of New Zealand falcons by people because they hunt domestic chickens and pigeons. This was first recorded by Fox (1977) but since regularly reported, throughout the winter months, even though the species has been fully protected since 1970. The IUCN classifies them as near threatened while the DOC conservation threat status (Robertson et al, 2013) differs for each morph, Southern

and Bush are “threatened (nationally vulnerable)” and Eastern are considered to be “at risk (recovering)”. The species is recorded as taxonomically indeterminate/ data deficient.

## **1.2 New Zealand falcon taxonomy:**

Falcons in New Zealand were first recorded by European observers in 1790 when Sir Joseph Banks mentioned in his account of New Zealand the presence of hawks and owls that were somewhat similar to the British species (Morrell, 1958). George Forster, painter on HMS Resolution, collected one specimen in 1773 on the same voyage, and painted it as part of his collection of paintings. Size dimorphism in the falcon was noted by Bayley in 1773 who saw two kinds of small hawks’ at Queen Charlotte sound (McNab, 1914). Whether they were different sexes, ages, morphs or species such as harrier and falcon is unknown. During this time the falcon amassed a number of names including *Falco novae-zeelandiae* (Gmelin 1788) and *Falco harpe* (Forster 1773) *Falco brunnea* (Gould 1837) and *Falco australis* (Hombr. et Jacq 1841). It is clear the morphism in the species caused much confusion in the species taxonomy. The question of whether New Zealand falcon comprised two separate species has been considered by several ornithologists.

Gray reported the presence of two types of falcon, labelling them as type A and type B (Dieffenbach, 1843). Smith (1884) recorded two species; a quail hawk and a bush hawk; that he said were found in different habitats. Sharpe (1873) working on skins in the British Museum was unable to resolve the question, except to determine that there may have been two groups. Walter Buller again attempted a solution in his *History of New Zealand Birds* (1888), reporting a large and small form. Moncrieff (1927) made a study of New Zealand falcon skins. She examined morphometrics and colouration and could not come to a firm conclusion, except that North Island birds were smaller than the South Island ones. Moncrieff stated that there was a large variation in size and a large overlap and concluded that she could not recognise two distinct species.



Overall studies appear to have had insufficient material to allow definite conclusions. Subsequent literature on New Zealand falcons avoided the taxonomic issue or only mentioned the possibility of more than one type, but did not classify more than falcon one species. Turbott (1967) concluded through Buller's work that there existed a single variable species.

Morphological data collected by Fox (1977) led to the proposal of three populations differing in wing and tail lengths, weight and colour. The Bush falcon's wings seem to increase slightly in size with latitude following Bergmann's rule. There is also a break in bush habitat along the west coast of the South Island, these two factors resulted in Fox (1977) breaking the Bush falcon into the Bush falcon and the Southern falcon, the Southern being intermediate in size between the Bush and Eastern falcon. The habitat for the Southern falcon contains open habitat such as grasslands in the higher altitude.

In general, Bush and Eastern falcons are a similar shape but the Bush falcon is much smaller and with a tendency to be darker. Fox (1977) discovered that Eastern falcons had on average a wing 20.9mm longer than Bush falcons. Southern falcons are an intermediate form, but more closely similar to the Bush than the Eastern (Fox 1977). If real, this might be influenced by habitat availability, Eastern falcons hunt and generally live in more open agricultural and high country areas compared to Bush falcons that hunt and nest in forest in the North Island and north west South Island. As a result nesting sites are often different with the Eastern falcon nesting on cliffs, bluffs and open ground and the Bush falcons using epiphytes and logs in the forest. Such inferences are however, generalizations, however, some Eastern falcons live in forest and Bush falcons are able to live on cleared land. Falcons are also now documented as living and breeding in exotic pine plantations (Ryder 1948, Stewart & Hyde 2004).

New Zealand falcons show many of the same behavioural characteristics as other falcon species which could contribute to the polymorphism evident within the species. Philopatric behaviour could reduce the capacity for gene flow among populations and regions and may also restrict the colonization ability over great distances (Alcaide et al,

2009). Fox (1977) concluded that adult New Zealand falcons remain in or near the territory of their birth all their lives and there is little or no evidence of former parts of the range being recolonized (e.g. Northland or the Chatham Islands). Other falcons such as the peregrine falcon (*Falco peregrinus*) usually return to within a few kilometres of their birth to breed even though they are capable of travelling great distances (White et al, 2013). Although peregrine falcons have dispersed widely as a species, individuals may have a limited tendency to colonize long distance especially in resident populations. For example peregrines have not colonised New Zealand despite proximity of populations to New Zealand. This may be due to their high philopatry, coupled with their solitary nature. Long term ringing studies of lesser kestrel (*Falco naumanni*) in Spain is documented as having a high level of natal and breeding philopatry. In contrast, the Eurasian kestrel (*Falco tinnunculus*) exhibit less intense philopatry in populations in northern and western Europe (Alcaide et al, 2009).

There are records and observations in the New Zealand falcon imprinting on nesting habitat which may contribute to philopatric behaviour (Fox, 1977). Of 11 pairs he studied for two or more years all chose the same type of nest site (rock ledge or under a log) in consecutive years. Based on a small number of banding returns and evidence of near permanent roosts (cake roosts), Fox suggested that males were attached to their nesting territory. He noted that at some cake roosts so many droppings had accumulated that they had blocked the roost from further use (Fox, 1977).

There is the possibility that ecological selection resulting from differences in the habitat type in which each morph lives has driven the differences in morphology. The putative geographic regions occupied by the different morphs represent different primary habitats. The South Island has vast open areas supporting the idea of the Eastern falcons using open ground hunting techniques benefited by large size, whereas the North Island and West coast of the South island is (or was until recently) primarily closed bush that is more easily hunted by smaller falcons. The smaller body size results in more efficient hunting in the dense under growth. Fox (1977) believed the Bush falcon maybe becoming more accipiter like in its hunting and morphology.

Hawks (Accipitridae) and falcons (Falconidae) tend to employ very different hunting strategies. With hawks typically using ambush tactics, with explosive tail chases, and ultimately extend their legs in front of their bodies to clutch prey with their feet. Falcons usually strike their prey from above at high speeds, with their feet opened and held firmly in position close to their body (Sustaita, 2008). The accipiter style suits forest living where dense undergrowth prevents striking from above at speed. Distinct habitat use could also enhance geographic isolation between the morphs, strengthening the opportunity to adapt to different niches. Differing habitats also support different prey types impacting the hunting style, nesting habitats and home range sizes.

### **1.3 Plumage Differences:**

The tail barring (number and colour) of juvenile and adult falcons and male and female falcons tend to be quite different (Fox, 1977). Gloger's rule states that animals living in warm and humid regions have darker pigmentation than those living in colder and drier regions (Gloger, 1883). Male falcons have more tail bars than females and Eastern falcons have significantly more tail bars than Bush falcons, possibly following Gloger's rule and the Southern falcon are closer to the Bush falcons in tail bar numbers (Fox, 1977). Primary feather bars consist of white stripes on the P8 (the longest primary) there is some difference in the numbers of bars, but the difference is small and insignificant (Fox, 1977). Body colouration shows some variation in colour of the dorsal surface, however, this can be ascribed to individual variation. It appears from Fox's (1977) analysis that the plumage variation displayed in New Zealand falcons may correlate with the three morphs proposed.

There are a number of issues in quantifying colour differences in bird species. There have been many studies conducted in regards to plumage differences in gyrfalcons (*Falco rusticolus*) and saker falcons (*Falco cherrug*) as well as in the peregrine falcon. There are at least 19 recognised subspecies of peregrine falcon, each apparently with distinct morphology and colouration. However, within each subspecies of peregrine

falcon, considerable variation in coloration has been described, especially at the limits of their distribution or in areas of potential overlap. The relationship between plumage variation and genetic variation is ambiguous. Barbary falcons (*Falco pelegrinoides*) are thought to be a sub species of peregrine but many believe it to be a separate species (Rodriguez et al, 2011). Genetically, barbary falcons appear to be very similar to other peregrine falcons but they are distinctive in size and colour (Rodriguez et al, 2011).

There is considerable variation in the group that suggest that colour provides a poor indicator of speciation (Rodriguez et al, 2011). Gyr falcons show an even greater degree of colour variation, ranging from white to black. Research show that the white/melanic colour pattern observed in gyr falcons is explained by allelic variation at the Melanocortin-1 receptor gene. Six nucleotide substitutions were observed in the gene resulting in nine alleles that differed in frequency throughout the gyr falcon's range of Greenland, Iceland, Canada and Alaska. The degree of melanism was correlated with variant alleles, with silver gyr falcons being heterozygous and the dark grey individuals homozygous. Plumage variation in the New Zealand falcons is not as extreme as the gyr falcons but variation is evident and further work could be done to determine the extent of variation and its correlation with other traits. How this relates to habitat is also an interesting subject for future research.

#### **1.4 Reverse Sexual Dimorphism:**

The use of simple morphometrics in the study of falcons is made difficult because of the reversed sexual dimorphism (RSD) present in the genus. In New Zealand falcons females are regularly recorded as being up to a third larger than the males, an occurrence that is recorded in a number of falcon species (table1.1). Because of this size dimorphism, all analyses in this research are conducted separately for males and females. Many studies have explored the evolutionary explanation for the large size difference in males and females. Among hawks, falcons and owls the strongest correlate with reversed sexual dimorphism was hunting method (Kruger, 2005).

Where there is role differentiation between the sexes, males and females can exploit different prey (Reynolds, 1972 & Newton, 1979). Interestingly, an evolutionary pathway analysis suggested strongly that the evolution of RSD preceded the specialisation on more agile and rarer prey. Hence RSD might have evolved for more efficient foraging (another important correlate was reproduction rate), fitting the small male hypothesis, which was followed by specialisation on more agile and rarer prey (Kruger, 2005). The small-male hypothesis may be the most likely general explanation for the evolution of reversed size dimorphism in falcons (Kruger, 2005). For example the male needs to be as efficient as possible during the breeding season because the female relies on him while laying and incubating eggs. While the New Zealand falcon sexes share incubation of the eggs, once the chicks hatch the female does the majority of the chick care. Kruger (2005) suggests that if small prey (e.g. house sparrows in New Zealand) is more abundant and easy to catch, the male will capture more during time away from the nest and spend less time searching. Whereas, when the female leaves the nest she needs to be away for the shortest time possible due to the possibility of nest predation so a larger size allows to her to catch a wide variety of prey and bring larger bundles of food to young.

Table 1.1: Mean male and female wing lengths for different falcon species and subspecies.

Species	Mean male	Mean female	Difference	Author
	wing length (mm)	wing length (mm)		
<i>Falco novaeseelandiae</i>	241	281	40	
<i>Falco peregrinus macropus</i>	287	325	38	Johansson et al (1998)
<i>Falco peregrinus tundrius</i>	308	348	40	Johansson et al (1998)
<i>Falco peregrinus minor</i>	284	321	37	Jenkins (1995)
<i>Falco mexicanus</i>	305	346	41	Steenhof & McKinley (2006)
<i>Falco biarmicus</i>	315	354	39	Jenkins (1995)
<i>Falco tinnunculus rupicolus</i>	246	256	10	Groombridge et al (2004)
<i>Falco punctatus</i>	173	187	14	Groombridge et al (2004)
<i>Falco columbarius</i>	265	294	29	Wiklund (1998)

## 1.5 Conclusion:

Understanding the relationship between phenotypic and molecular genetic data sets may be particularly informative in studies of avian taxonomy where plumage characteristics have been historically used to designate subspecies as well as species. Many species and subspecies designations based upon phenotype alone have not been supported by molecular genetic phylogenies (Hull et al, 2010). Inconsistencies at both hierarchical levels are associated with the difficulty in understanding the underlying genetic and/or environmental basis of phenotypic characters, and consequently, interpreting phenotypic patterns alone may mislead evolutionary inference (Hull et al, 2010). Phenotypic characters associated with melanin-based plumage may be particularly susceptible to misinterpretation in an evolutionary context due to environmental and temporal influences (Griffith et al, 2006) which is quite possible in the New Zealand falcon, where observation suggests New Zealand falcons from bush habitats are much darker than those elsewhere.

Without proper classification the New Zealand falcon cannot have an appropriate management plan in place. Estimating genetic divergence among populations is crucial for the conservation of many threatened and endangered species (Allendorf & Luikart, 2007). Many management programs are concerned with resolving phylogenetic and taxonomic uncertainties among taxa in order to prioritize conservation efforts below the species level (e.g. subspecies, evolutionary significant units). This is important because much existing taxonomy may not reflect the underlying genetic diversity (Hass et al, 2009). Unfortunately, basic information on population genetic divergence is lacking for many threatened species, hindering conservation and management actions such as translocations and reintroductions (Moritz, 1999). The New Zealand falcon is one such species where such basic information is unavailable and this makes it impossible to create an appropriate management plans for the species. My research will inform the management of New Zealand falcon. In particular, it will indicate if the three morphs should be managed as separate taxonomic units/sub species or as one species. There is no current management plan in place for the New Zealand falcon; this

research will provide information on the population structure and thus a basis for a management plan. It will also assist in decisions about future translocations and reintroductions of individual falcons.

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## Chapter 2:

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### **Phylogeny of the *Falco* genus: where does the New Zealand falcon sit?**



Female New Zealand falcon, Awatere River Mouth, Marlborough (Photo-Lena Olley)

## 2.1 Introduction:

### 2.1.1 The *Falco* genus:

Among birds, the raptors display some of the most advanced aerial flying skills and exploit the capacity to fly in their daily lives. Recent phylogenetic evidence indicates that the raptorial lifestyle has evolved many times. Diurnal raptors are placed in the order Falconiformes, and are usually grouped into five families, Accipitridae (hawks, kites, buzzards, eagles and old world vultures), Pandionidae (Osprey), Sagittariidae (Secretary bird), Falconidae (falcons, caracaras and falconets) and Cathartidae (new world vultures and condors) (Wink & Sauer-Gurth, 2004). However, the order Falconiformes is an artificial unit which combines birds that share a common lifestyle, especially behaviour and ecology. Recent research suggests Cathartidae, Falconidae and Sagittariidae do not share direct ancestry with Accipitridae and Pandionidae (Wink et al, 2004). Falconiformes share behavioural and ecological traits with owls (Strigiformes), which are a separate order altogether, further questioning the relevance of taxonomic classification of raptors by life history alone.

It is difficult to know which extant genera within the family Falconidae are oldest or most 'primitive'. The caracaras, laughing falcons and forest falcons still possess what are considered ancestral characteristics representing the descendants (Cade, 1982). It is logical to assume the forest dwelling or adapted species preceded the true falcons. If this logic is sound, then South America may be where the falcon originated. However what happened after this and how they expanded globally is harder to define.

Falcons (genus *Falco*) sit inside the family Falconidae and are small to medium sized, compact, long winged, diurnal birds of prey, all of which are included in the single genus, *Falco*. They range in size and variety from the brightly coloured, wind hovering kestrels, often weighing less than 100 grams to the large, monochrome, arctic Gyr falcons, some of which weigh more than two kilograms (Cade, 1982). Where the genus fits within the order Falconiformes is debated, however, it is generally accepted that

the *Falco* lineage is most closely related to the caracaras, laughing falcon, forest falcons and pygmy falcons (figure 2.1).

The *Falco* genus may have split from the other species within the Falconidae family approximately 12 million years ago (Fuchs et al, 2011) during the mid-Miocene. The oldest record of the genus *Falco* is from a fossil found in South Ukraine dated to the late Miocene (Boev, 2011). It is possible that during the late Miocene and early Pliocene, there was rapid diversification among the genus once the combination of characters adapting the birds to aerial hunting in open habitats had been optimised by natural selection (Darlington 1957, Cade 1982).

The fossil history of falcons is poor, with approximately 10-11 species described so far, some of which aren't considered valid species (Boev, 2011). Boev (2011) suggests that the genus *Falco* split into two main groups ('tinnunculus'- smaller falcons and 'cherrug' – larger falcons). The 'tinnunculus' group appears to have diverged earlier than the 'cherrug' group. A species of small falcon placed in 'tinnunculus' dates from the late Miocene, while 'Cherrug' records begin to appear in the early Pleistocene (Boev, 2011). This is supported by molecular data placing the emergence of peregrine falcons (*F. peregrinus*) in the late Pliocene or early Pleistocene (White et al, 2013).

Morphologically, members of the *Falco* genus are a very homogenous group of species which makes it difficult to assess phylogenetic relationships within the genus (Cade, 1982). Interest in solving this relationship has been around for over 100 hundred years since publications by Suschkin (1905) and Klienschmidt (1901). Most attempts to resolve *Falco* systematics were made through analysis of morphological and plumage characteristics. Fox (1977) proposed two waves of adaptive radiation resulting in the modern diversity of the falcons. Firstly, the species exhibiting forest falcon type traits spread throughout the tropics, but failed to successfully establish in Australia. A second radiation of falcons and falconets then occurred as climatic and ecological changes reduced the extent of tropical forests and greatly expanded grassland (Fox, 1977). This hypothesis is supported in a phylogenetic analysis by Wink (2002)

concluding that the new world falcons diverged longer ago than the old world falcons, and that the American kestrel (*F. sparverius*) occupies a basal position in a in the tree. This further supports the idea of an ancestral forest falcon mentioned earlier.

Other hypotheses suggest two centres of falcon diversification with Gondwanan and African origins (Olsen et al, 1989). Africa does have more species of falcon than any other continent, and many species appear to have had their origin there such as the fox kestrel (*F. alopex*), the desert falcons and merlins. Olsen (1989) also suggests that some falcon species such as the New Zealand falcon originated and diversified in the southern hemisphere. Feather protein analysis showed that this group including the bat falcon, sooty falcon, Eleonora's falcon and brown falcon had a character also seen in pygmy falcons (*Polihierax spp*), indicating that the group may have an earlier origin than other falcon groups (Olsen et al, 1989). However this was mostly accepted as being unlikely, and most of the knowledge and research on the genus *Falco* seems to indicate a single Gondwanan origin. The family Falconidae show a rather strong biogeographic genetic signal (Griffiths et al, 2004). Two of three strongly supported basal clades appear to be restricted to the Neotropics while the third clade, positioned so as to render the first two paraphyletic, was worldwide in its distribution. Thus the falcons are a Neotropical group that has secondarily expanded globally (Griffiths, 2004).

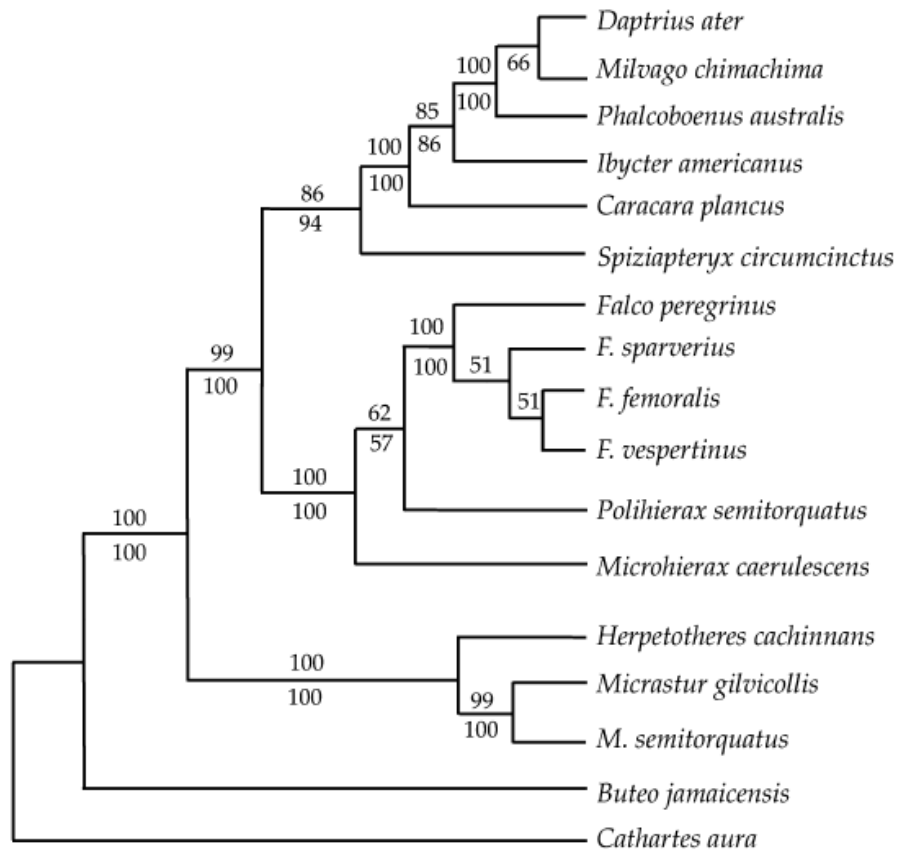


Figure 2.1: Relationships within the family falconidae with the turkey Vulture (*Cathartes aura*) as an out group, taken from Griffiths et al (2004) using molecular RAG -1 data. The *Falco* genus is closely allied with pygmy falcons and falconets. *Micrastur gilvicollis* and *Polihierax semitorquatus* were used as out groups in our analysis.

There are many uncertainties surrounding *Falco* taxonomy, with many subspecies proposed. There have been up to 40 species of falcon described; but generally 37-39 species is the accepted consensus (table 2.1). Various studies have considered relationships amongst the genus *Falco*. Olsen (1989) includes 37 species in her analysis of feather proteins, while Cade recognises 39 species adding in two possible morphs of the peregrine as separate species (Cade, 1982). Numerous subspecies have been proposed within the *Falco* genus. Peregrine falcons are the most widely distributed falcon species found across globe except in places such as New Zealand and the Antarctic. They vary in morphology throughout their range, this variation, largely in plumage characteristics but also in meristic measurements, has led to the description of over 75 subspecies, with as many as 19 subspecies of peregrine falcons currently recognized (White et al, 2013).



Falcons are generally accepted to fall into six distinct adaptive types based mostly on morphology and habits (Olsen et al, 1989). These are:

1. Typical kestrels (subgenus *Tinnunculus*) - This includes the Australian/nankeen kestrel, common/Eurasian kestrel, moluccan kestrel, Madagascan kestrel, Mauritius kestrel, Seychelles kestrel, lesser kestrel, fox kestrel, greater kestrel & American kestrel.
2. Aberrant kestrels (subgenus *Dissodectes*) - grey kestrel, Dickinson's kestrel, barred/banded kestrel and possibly the red footed falcon and amur falcon.
3. Merlins (subgenus *Aesalon*) - merlin and red headed/necked falcon.
4. Hobbies (subgenus *Hypotriorchis*) - Australian hobby/little falcon, African hobby, oriental hobby, Eurasian hobby and possibly the sooty and Eleonora's falcon.
5. Peregrines - peregrine falcon and barbary falcon plus other subspecies, the taita falcon is also proposed as belonging in this group (Brown and Amadon, 1968).
6. Desert falcons (subgenus *Heirolfalco*) - prairie falcon, gyr falcon, lanner falcon, laggar falcon and saker falcon. It is thought the black falcon may also belong here (Wink 2004, Fox 1977). Opinions are mixed as to whether to place peregrines in this subgenus, sometimes it is included, sometimes placed separately.

However, there are a number of species of which their placement in the group is largely unknown, mostly those species from South America, Australia and New Zealand. While there are a lot of opinions on the matter, no firm conclusions have been made.

Table 2.1: List of 39 falcon species, authorities (Birdlife International) and distribution, includes two peregrine morphs recognised as separate species by Cade, 1982 (*F.pelegrinoides* and *F. kreyenborgi*). Grey shading includes generally accepted species of the subgenus *Heirofalco* while light blue indicates those species included in the 'kestrel' group.

Common name	Latin name (Authority)	Distribution
Aplomado falcon	<i>Falco femoralis</i> (Temminck,1822)	South America
Orange breasted falcon	<i>Falco deiroleucus</i> (Temminck,1825)	South America
Bat falcon	<i>Falco ruficularis</i> (Daudin,1800)	South America
Brown falcon	<i>Falco berigora</i> (Vigors & Horsfield,1827)	Australia
Little falcon	<i>Falco longipennis</i> (Swainson,1837)	Australia
Grey falcon	<i>Falco hypoleucos</i> (Gould,1841)	Australia
Black falcon	<i>Falco subniger</i> (Gray,1843)	Australia
Peregrine falcon	<i>Falco peregrinus</i> (Tunstall,1771)	Asia, Africa, Europe, America, Australia
Gyr falcon	<i>Falco rusticolus</i> (Linnaeus,1758)	Circum polar
Lagger falcon	<i>Falco jugger</i> (Gray,1834)	Asia
Lanner falcon	<i>Falco biarmicus</i> (Temminck,1825)	Africa, South East Europe & Asia
Saker falcon	<i>Falco cherrug</i> (Gray,1834)	Europe and Asia
Barbary falcon	<i>Falco pelegrinoides</i> (Temminck,1829)	Asia, Africa
Pallid falcon	<i>Falco kreyenborgi</i> (Kleinschmidt,1929)	South America
Prairie falcon	<i>Falco mexicanus</i> (Schlegel,1850)	North America
Red-necked falcon/merlin	<i>Falco chicquera</i> (Daudin,1800)	India & Africa
Amur falcon	<i>Falco amurensis</i> (Radde 1863)	Asia
Sooty falcon	<i>Falco concolor</i> (Temminck,1825)	North Africa, Persian gulf
Eleonoras falcon	<i>Falco eleonora</i> (Gene,1839)	Mediterranean Islands
Taita falcon	<i>Falco fasciinucha</i> (Reichenow & Neumann,1895)	Africa
Red footed falcon	<i>Falco vespertinus</i> (Linnaeus,1766)	Eastern Europe, Africa & Asia
New Zealand falcon	<i>Falco novaeseelandiae</i> (Gmelin,1788)	New Zealand
Nankeen kestrel	<i>Falco cenchroides</i> (Vigors & Horsfield,1827)	Australia
Common kestrel	<i>Falco tinniculus</i> (Linnaeus,1758)	Europe, Asia, Africa, North America
Lesser kestrel	<i>Falco naumanni</i> (Fleischer,1818)	Asia
Mauritius kestrel	<i>Falco punctatus</i> (Temminck,1821)	Mauritius
Malagasy kestrel	<i>Falco newtoni</i> (Gurney,1863)	Madagascar
Seychelles kestrel	<i>Falco araea</i> (Oberholser,1917)	Seychelles
Spotted kestrel	<i>Falco moluccensis</i> (Bonaparte,1850)	Australia, Malaysia
Greater kestrel	<i>Falco rupicoloides</i> (Smith,1829)	Africa
Fox kestrel	<i>Falco alopex</i> (Heuglin,1861)	Africa
Grey kestrel	<i>Falco ardosiaceus</i> (Viellot,1823)	Africa
Dickinsons kestrel	<i>Falco dickinsoni</i> (Sclater,1864)	Africa
American kestrel	<i>Falco sparverius</i> (Linnaeus,1758)	America
Barred/Banded kestrel	<i>Falco zoniventris</i> (Peters,1854)	Africa
African hobby	<i>Falco cuvierii</i> (Smith,1830)	Africa
Oriental hobby	<i>Falco severus</i> (Horsfield,1821)	India
Eurasian hobby	<i>Falco subbuteo</i> (Linnaeus, 1758)	Africa, Europe, Asia
Merlin	<i>Falco columbarius</i> (Linnaeus,1758)	Northern hemisphere

### 2.1.2 New Zealand falcon placement:

As with most falcon species the taxonomy of the New Zealand falcon is difficult. Over time it has been described using up to 15 different Latin names, much of the controversy has been due to its size dimorphism (Fox, 1977). Early taxonomic work on this was based mainly on skeletal features, moult patterns, behaviour and general morphology. Suschkin (1905) believed the New Zealand falcon possessed many so called 'primitive' features in its skeletal system. The 'primitive' characters he spoke of included: a relatively small cranium, poorly developed occipital processes and a relatively long head. He considered that the New Zealand falcon had evolved unique features in isolation, but suggested they were similar to hobbies in their orbital processes and the skeleton of the feet and legs. The merlin (*F. columbarius*) and red-headed falcon (*F. chicquera*) were placed in the merlin group (*Aesalon*) separate from the kestrels but branching earlier than hobbies so Suschkin (1905) put New Zealand falcons and *Aesalon* closer together than the other species.

Stegmann (1933) noted that New Zealand falcons had a laterally compressed bill with a sharp dorsal ridge and that this character was confined to New Zealand falcons, orange breasted falcons (*F. deiroleucus*), bat falcons (*F. ruficularis*) and aplomado falcons (*F. femoralis*) (of which, apart from the New Zealand falcon are all from South America). These species shared atypical tail bars that consist of narrow white bars on a dark background. This is not seen in any other species apart from in more 'primitive genera' such as forest falcons (*Micrastur* spp). Brown and Amadon (1968) suggested that the New Zealand falcon and the Australian brown falcon are both aberrant hobbies and are related to the Australian hobby. This appears mostly to rely on the geographical distribution.

Fox (1977) suggests that the New Zealand falcon's closest relative maybe the orange breasted falcon and that it shares a close relationship to the bat falcon and the aplomado falcon. Together, he considered that the New Zealand falcon and these three South American falcons formed a closely related subgroup. Fox (1977) also

concluded that the New Zealand falcon has evolved convergently to be more similar to the accipters than have other members of the genus *Falco*. He placed the South American falcons in their own group *Nesierax* along with the New Zealand falcon (the subgenus *Nesierax* was first proposed by Stegmann (1933)). These species are different in their ecology and in their morphological adaptations for catching prey. The orange breasted falcon has large feet like a peregrine, while the bat falcon is more hobby-like. The aplomado and New Zealand falcons are both more accipter like. However, like Stegmann (1933), Fox (1977) noted the similarities within the group, including a laterally compressed bill with a distinct dorsal keel and similar plumage patterns, and explained these similarities by suggesting that there existed a Gondwanan group of falcons.

The first molecular work on the New Zealand falcon began in the 1980's. Olsen et al (1989) explained that based on feather protein analysis the New Zealand falcon and brown falcon (*F. berigora*) of Australia appear to be closely related. Olsen et al (1980) suggest that the brown Falcon can be thought of as relatively primitive, but is not close to the kestrels, and should be placed with the New Zealand Falcon as a two-member component of a cluster of 'Gondwanan' falcons. The second component of this cluster should include the Eleonora's, (*F. eleonora*) sooty (*F. concolor*) and bat Falcons and all appears to have originated and diversified in the Southern Hemisphere (Olsen et al, 1989). Wink (2004) used nucleotide sequence data which put the New Zealand falcon as being closely related to the Dickinson's kestrel (*F. dickinsonii*) from East Africa. However, this analysis was uncertain, and Wink (2004) could not draw a strong conclusion. While other molecular work has been completed on the *Falco* genus, very few studies have included the New Zealand falcon, and most proposals surrounding its taxonomy are therefore based on morphology.

### **2.1.3 Aims:**

We aim to create a clearer picture of the relationships within the genus *Falco* and to gain a better understanding of where the New Zealand falcon may sit within it. We

want to understand this placement with regards to the current proposals of its placement, for example where it may sit in conjunction with the Australian and South American falcons.

## **2.2 Methods:**

### **2.2.1 Markers used:**

I used data from one mitochondrial gene and one nuclear gene. The primary data were comprised of sequences of mtDNA Cytochrome-b (Cyt-b) and nuclear RAG-1, which have both been shown to provide sufficient variation to resolve population structure in birds and have been used in phylogenetic analysis for other falcon species. CO1 data was also investigated; however, there were far fewer species of falcon with sequences available for this gene and so not used in this analysis. All available sequences for the *Falco* genus were retrieved from Genbank (NCBI). There are a number of factors that make DNA sequences superior to any other kind of data for assessing phylogenetic relationships among species. The variability of the DNA is much higher than at the protein level because the genetic code is redundant. Therefore, most base substitutions that occur in protein coding regions are so called silent substitutions, and cannot lead to convergent evolutions (Griffiths, 1997). Most recent DNA studies on avian phylogeny have used the mitochondrial genome (mtDNA), mainly for two reasons. MtDNA is about 10 times more variable than nuclear DNA in higher vertebrates, and it's inherited clonally and not subject to recombination. Within the mtDNA genes the cytochrome-b (Cyt-b) sequence is well characterized and is now becoming a standard reference for phylogenetic and taxonomic studies in birds. It has a relatively high rate of base substitution, a high availability of near universal primers and a large published dataset for comparison throughout the avian group (Griffiths, 1997). It has also been found to be sufficiently variable to describe 17 taxa of the *Falco* genus (Helbig et al, 1994). The single-copy RAG-1 gene is found throughout higher vertebrates and consists of a single 3.1-kb exon (Groth & Barrowclough, 1999). The

RAG-1 gene has many properties desirable for molecular phylogenetic analyses, indels are rare, and the few that exist do not cause alignment or homology problems. Like the Cyt-b gene it had been used in a number of studies providing a large dataset for comparisons.

### **2.2.2 Sample collection:**

Samples from blood, feathers, muscle and toe tissue were collected from various sources and locations. Samples from New Zealand falcons originated from throughout New Zealand except north of Auckland. Sources included The Department of Conservation, museums, captive facilities, universities, individual researchers and bird enthusiasts. Samples were taken from museum specimens included preserved birds collected following accidental deaths and from feathers and blood taken from both wild and captive birds. Tissue samples collected from muscle and toe pads were preserved in 90% ethanol. Feathers were stored dry in plastic bags and frozen. Blood samples were stored in either 90% ethanol or Seutin's buffer and then frozen. Samples were also taken from five Australian falcon species. Samples from the little falcon, brown falcon, black falcon and the nankeen kestrel were provided by the Australian National Wildlife Collection (CSIRO Ecosystem Sciences) in Canberra. Samples from the grey falcon were provided by the Australian Museum in Sydney.

### **2.2.3 DNA Extraction:**

Museum specimens were sampled by taking a small scraping (approximately 3mm by 3mm) from the toe-pad using a sterile scalpel. Feathers were sampled by removing 2–3mm of the tip of the feather. Approximately 50-100 µm of blood was taken out of the buffered samples. A 3mm by 3mm section of muscle tissue was taken from the chest of frozen whole birds. Extraction methods were selected and modified as appropriate for the type of tissue/sample used. Experiments were conducted to determine the best method for each tissue type. A Phenol/chloroform method was used for feathers

and either GenElute kit (Sigma-Aldrich) or Phenol/chloroform method was used for blood, toe pads and muscle tissues.

All samples were digested through incubation at 55 C ° with Proteinase K (10ng/μl) and a CTAB buffer(100ml 1M Tris, 280 ml 5 M NaCl, 40 ml of 0.5 M EDTA and 20g of CTAB) in a Total Lab Systems LTD Infors Minitron shaker at approximately 200rpm for 10-24 hours until digested. This was followed by a combined phenol, chloroform/isoamyl alcohol clean-up based on methods previously described (Sambrook et al, 1989). DNA was precipitated using 500μl of ice-cold ethanol, mixed via inversion and left in freezer overnight. After spinning at 14,000rpm for 10min, the ethanol was poured off, and after drying the DNA was re-suspended in 40μl of H<sub>2</sub>O for storage. The concentration and quality of DNA was checked using a Thermoscientific nanodrop spectrophotometer and ND-1000 V3.6.0 software. GenElute extractions were performed following manufacturer's instructions. The extracted DNA was stored in a minus 20°C freezer.

#### **2.2.4 PCR Amplification:**

PCR amplifications were carried out in 10 microliter volumes using Mclab taq or Roche taq. Each gene required a different PCR protocol for the best results. Roche taq was used for RAG-1 reactions, while Mclab was used for Cyt-b reactions. Recipes for the 10μl PCR reactions were as follows: 0.5 μl reverse and forward primers, 1 μl manufacturer's buffer, 1 μl dNTP's, 5.9 μl H<sub>2</sub>O, 0.1μl MClab or Roche taq. When using the Roche taq/buffer 2 μl of magnesium was added. All PCR products were checked on 1% agarose gels. We sequenced two fragments of the mitochondrial Cyt-b gene each 1135 base pairs long. As this fragment could not be amplified in its entirety, two primer sets were used (table 2). Internal primers 15107 and 15307 were used in combination with AV14857 and 16065falco respectively, to amplify two overlapping fragments covering the same portion of the gene then Sequencher (v 5.2.4) (Gene Codes Corporation) was used to form a consensus sequence. The protocol set for

amplification was as follows: an initial denaturing of 94°C for 3 min followed by a cycle of 94°C for 30 sec, a 15 sec annealing period set at 50°C and a 45 sec extension at 72°C, finished by a 5 min final extension set at 72°C. Two sets of primers were also used to amplify the RAG-1 sequence in two parts using primers R50 (Irestedt et al, 2001) and R2b (Groth & Barrowclough, 1999) in conjunction with R53 (Irestedt et al, 2001) and R13b (Groth & Barrowclough, 1999) (see table 2.2). The amplification protocol was as follows an initial denaturing of 94 C° for 5 min followed by four cycles of 94°C for 40sec, an annealing period at 63°C for 1 min then 72°C for 1min. A second set of 4 cycles was then performed at 94°C for 50sec, with an annealing period at 60°C for 1 min then 72°C for 1 min, a third cycle followed for 32 cycles of 94°C for 40 sec, then an annealing of 55°C for 1 min, then an extension of 72°C, and a final extension of 72°C for 5 min using a Biometra T3000 thermo cycler. Concentration and quality of DNA was checked using a Thermoscientific nanodrop spectrophotometer and ND-1000 V3.6.0 software. Concentration and quality were also checked by electrophoresing DNA on a 1% agarose gel (Amesco agarose powder and 1x TAE Buffer: 40 mM Tris acetate, 1mM EDTA pH 8.0) alongside a 1Kb+ DNA Ladder (Life Technologies). The gel was run at 90 V for one hour. DNA was visualised and photographed under U.V. light using a BioRad gel-doc with BioRad Quality One 4.4.0 software. Concentration information was used to dilute DNA for each specimen to an approximately 10ng<sup>-1</sup> µl working solution. The sequencing reactions were performed by the Massey University Genome Service Center using a capillary ABI3730 automated Genetic analyser (Applied Biosystems).

Table 2.2: Primer names and sequences used in PCR reactions for Cyt- b and RAG-1 genes.

Gene	Primer name	Primer sequence
Cyt-b	AV14857	GGGTCTTTCGCCCTATCAAT
	16065falco	TYGGYCTTCATTCTTTGGTTTACAAGAC
	15107	CATCCGTTGCCACACATGYC
	15307	CAGTTGCTCCTCAGAATGAT
RAG-1	R2b	GAGGTATATAGCCAGTGATGCTT
	R13b	CTCCCTGAAGAGATTCAGCATCC
	R50	CTGATCTGGTAACCCAGTGAAATCC
	R53	TCCATGTCTTTAAGGCACA



## 2.2.5 Analysis:

Sequences and alignments were checked using Sequencher (v 5.2.4) (Gene Codes Corporation) and Geneious (v 6.1.8) (Biomatters limited). Sequencher (v 5.2.4) (Gene Codes Corporation) was used to construct consensus sequences for both RAG-1 and Cyt-b sequences for the New Zealand falcon and for the five Australian species. Two sequences for the New Zealand falcon were also retrieved from the Genbank (NCBI) database, making a total of four New Zealand falcon sequences used in each analysis. Sequences of 32 other species of falcon were also retrieved from Genbank (NCBI).

Table 2.3: Type of analysis and inclusion of sequences and taxa for each gene (Australian sequences refer to the 500 base pair sequences I amplified).

	Cyt-b		RAG-1	
	Outgroup	No Outgroup	Outgroup	No Outgroup
<b>Mr Bayes Analysis</b>	√	x	√	√
<b>RAXML Analysis</b>	√	√	√	√
<b>Australian sequences included</b>	√	√	x	x

Phylogenetic analyses used the CIPRES Science Gateway (81) to implement RAXML(RAXML-HPC2 on XSEDE(8.0.0)) and MrBayes analyses (MrBayes 3.2.2 on XSEDE). All trees were constructed with one full alignment containing all possible species and individuals regardless of sequence length. Analyses were then repeated both with and without an out group and with the short sequences from the five Australian species taken out. This was done to determine the best methods for the tree construction. From these results each gene was then treated slightly differently, as they each vary in the amount of variation discovered in the alignments (see table 2.3).

RAG-1 sequences were aligned in Geneious (v 6.1.8) (Biomatters Limited) then exported as Phylip or Nexus files into CIPRES Science Gateway. The RAXML phylogenetic tree inference using maximum likelihood/rapid bootstrapping was run on the XSEDE programme. The first analysis used all 31 species and the lined forest falcon

*Micrastur gilvicollis*) as an out group; this was then repeated taking out the short Australian sequences. These were 500 base pairs shorter than the majority of other sequences and due to the low variation within the RAG-1 sequences generally they were thrown out in a group together in the RAxML analysis rather than with the right species group. The final analysis had these taken out because they could not be placed correctly; four species apart from the grey falcon were still represented by sequences from Genbank (NCBI). This process was repeated with MrBayes analysis (tree inference using Bayesian Analysis run on XSEDE). The resulting output files were then put into Splitstree (v 4) using the consensus network method to construct the tree. Bootstrap values above 50% have been included in the tree, these include values taken from analyses completed without an out group, where values increased support for certain clades.

The Cyt-b analysis used the same analysis as RAG-1 but because Cyt-b appeared to have a lot more variation in the alignment, it meant the shorter sequences from the Australian species were placed with the correct species groups in the tree, meaning these sequences were kept in for the final analysis. The out group included for this analysis was the pygmy falcon (*Polihierax semitorquatus*). Again, bootstrap values above 50% have been included on the tree and bootstrap results from the analyses completed with the out group excluded have been placed onto the trees with an out group included when they increased the support for certain clades.

## 2.3 Results:

For each gene two sequences from the New Zealand falcons were obtained, the Cyt-b fragment for the New Zealand falcon was 1135 base pairs long, while the 32 other species had sequences ranging from 900 to 1130 base pairs long. The grey falcon was 427 base pairs long, (a longer sequence could not be obtained in the time allotted due to poor DNA quality); while the four other Australian species extracted were only 578 base pairs long. RAG-1 sequences for the New Zealand falcon were 1562-1654 base pairs long; all of the other 29 species were 1770 base pairs long. While I did not include the shorter Australian sequences in the RAG-1 analysis, the four species (nankeen kestrel, brown, black and little falcon) were represented by sequences retrieved from Genbank (NCBI).

The exclusion of an out group from the analysis made little difference to the outcome or groupings of the main clades, and it also increased bootstrap support for many of the main clades (figures 2.2 & 2.3). It is important to note that in all trees most species groups also contained multiple subspecies and individuals that are shown, but not labelled on the tree. For example, peregrine falcons show multiple subspecies in the analysis, all of which were grouped together under *F. peregrinus/pelegrinoides* and the American kestrel had five individuals grouped together under the single name.

Each gene resulted in a slightly different tree construction due to Cyt-b having a higher number of variable sites than RAG-1. This resulted in a clear difference in the resolution between the two genes used in this analysis. RAG-1 has little variation in the gene (as observed in alignment in Geneious (Biomatters Limited)) making it difficult to construct a clearly resolved tree and resulting in an unresolved polytomy (Figure 2.3). This difference can also be seen where individuals of the same species don't get placed with other members of the same species for example the saker falcon getting split into three branches in the RAxML generated tree. The Cyt-b analysis doesn't seem to encounter the same problems because the gene has more variation and therefore

clearer trees with more defined species boundaries (Figure 2.2). Possible explanations for this are investigated in the discussion.

The analysis suggests that within the genus *Falco* there is an abrupt radiation resulting in a group of closely related species, this pattern can be seen in both analyses. However, two monophyletic groups are supported by both the Cyt-b and RAG-1 analysis, the *Heirolfalco* subgenus (9 species) and the 'kestrel' group (8 species). The other 16 species of falcon analysed relationships are more difficult to define. The structure seen in the trees don't show a clear biogeographic pattern with all clades including members from different parts of the world.

### **2.3.1 *Heirolfalco*:**

The subgenus *Heirolfalco*, or large desert falcons, are shown clearly as monophyletic in all analyses with both Cyt-b and RAG-1 data. The species included in this group are the gyrfalcon (*F. rusticolus*), saker falcon (*F. cherrug*), laggar falcon (*F. jugger*), lanner falcon (*F. biarmicus*) and the black falcon (*F. subniger*), all of which are generally accepted as belonging in the *Heirolfalco* subgenus in the literature (Helbig et al 1994, Siebold et al 1993, Olsen et al 1989 & Wink 2004.) The group also includes the peregrine falcons, *F. peregrinus* and *F. pelegrinoides* and all of its associated subspecies. Although scientists are divided on whether peregrines are included in the *Heirolfalco* subgenus, this analysis supports their inclusion but suggests they group together as a distinct species complex. The prairie falcon (*F. mexicanus*) and the grey falcon (*F. hypoleucus*) also belong in this group according to the Cyt-b analysis (figure 2.2).

### **2.3.2 Kestrels:**

Of the ten 'typical' kestrel species included in these analyses (there are thirteen recognised within the literature) all but the American kestrel (*F. sparverius*) and the Dickinsons kestrel (*F. dickinsoni*) grouped together in the Cyt-b analysis. The species

included in this monophyletic group in this analysis are the barred kestrel (*F. zoniventris*), usually considered an 'aberrant kestrel', lesser kestrel (*F. naumanni*), greater kestrel (*F. rupicoloides*), Malagasy kestrel (*F. newtoni*), Seychelles kestrel (*F. araea*), Eurasian kestrel (*F. tinnunculus*), nankeen kestrel (*F. cenchroides*) and Mauritius kestrel (*F. punctatus*).

### **2.3.3 The apparent hobby group and other tricky ones:**

The structure within the Hobby group (subgenus *Hypotriorchis*) is very unclear, with some support for the group mentioned in the introduction (Australian hobby/little falcon, African hobby, oriental hobby, Eurasian hobby and possibly the sooty and Eleonora's falcon) in the Cyt-b analysis but with the inclusion of the orange breasted falcon (*F. deiroleucus*) (figure 2) . However, the same pattern is not present in the RAG-1 analysis. Instead we see a group formed including the brown falcon (*F. berigora*), the Australian Hobby (*F. longipennis*), the amur falcon (*F. amurensis*) and the Red headed falcon (*F. chicquera*) (figure 3). It seems the red necked falcon (*F. chicquera*) could be placed in either the hobby or heirofalcon group and the red footed falcon (*F. vespertinus*) and amur falcon (*F. amurensis*) appear to be sister species. Over all, these species placements within this group are confounded due by the inconsistencies between each analysis.

### **2.3.4 New Zealand falcon:**

The relationship the New Zealand falcon has to other species in the *Falco* genus still remains quite unclear. The New Zealand falcon repeatedly falls by itself in all analyses; but is closer to the kestrel group than any other groups according to Cyt-b data (figure 2.2). The RAG-1 data suggests a relationship with the sooty falcon (*F. concolor*) (figure 2.3). The New Zealand falcon doesn't appear to have a sister or closely related species link to either an Australian or South America species as proposed in earlier research. Neither does it appear to fit within in any major clade like *Heirofalco* or the kestrels.

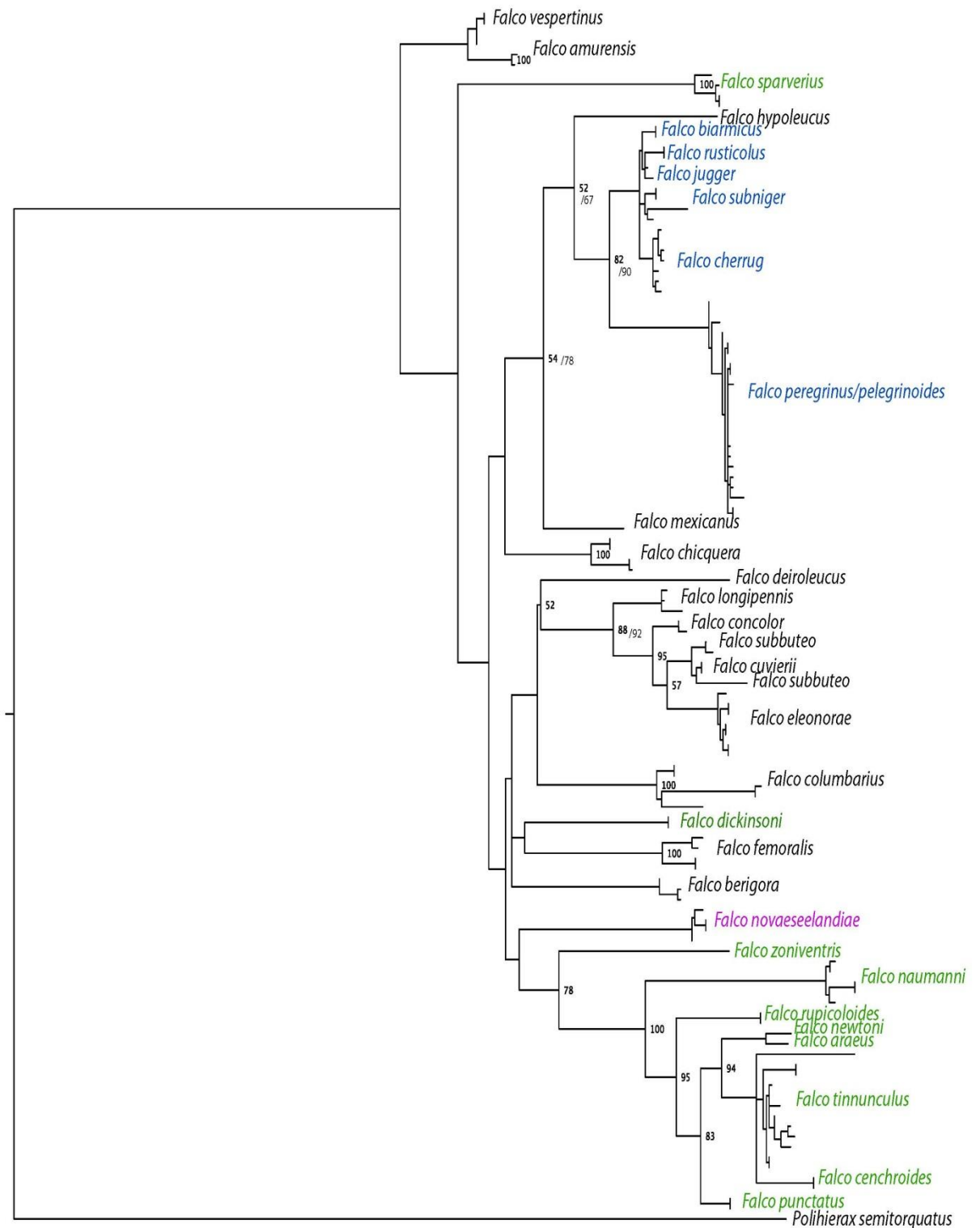


Figure 2.2: Phylogenetic relationships among 33 falcon species based on RAxML analysis using Cyt-b sequences and *Polihierax semitorquatus* as an out group. The New Zealand falcon (*Falco novaeseelandiae*) is highlighted in purple. Green text indicates the species of the accepted kestrel group (including 'typical' and 'aberrant' kestrels) and the blue text highlights the proposed members of the subgenus *Heirofalco* (*F. cherrug*, *F. rusticolus*, *F. biarmicus*, *F. jugger* and *F. subniger*) and *F. peregrinus* (including subspecies and *Falco pelegrinoides*). Bootstrap values of 50% or higher are included.



Figure 2.3: Phylogenetic relationships among 31 falcon species based on RAXML analysis using RAG-1 sequences and *Micrastur gilvicolis* as an out group. The New Zealand falcon (*Falco novaeseelandiae*) is highlighted in purple. Green text indicates the species of the accepted kestrel group (including ‘typical’ and ‘aberrant’ kestrels) and the blue text highlights the proposed members of the subgenus *Heirolfalco* (*F. cherrug*, *F. rusticolus*, *F. biarmicus*, *F. jugger* and *F. subniger*) and *F. peregrinus* (including subspecies and *F. pelegrioides*). Bootstrap values of 50% or higher are included.

## **2.4 Discussion:**

### **2.4.1 Phylogenetic placement of the New Zealand falcon:**

The New Zealand falcon has an obscure placement; first of all my results confirm that it is a falcon and is placed within the *Falco* genus. It does not appear to be sister to or closely related to any other single species in South America or Australia. Even though it appears to be ecologically and behaviourally similar to South American species such as the aplomado falcon (*F. femoralis*) (Fox, 1977) our analysis suggests that the aplomado falcon seems to fall with Dickinson's kestrel (*F. dickisonii*) in the Cyt-b analysis (also seen by Wink, 2002). Olsen (1989) concluded that the bat falcon (*F. ruficularis*) may be close to the Eleonora's falcon (*F. eleonora*) and sooty falcon (*F. concolor*) based on feather protein analysis. For a set of species which live so close together in their natural range it seems unusual to not have a close relationship genetically. However this is a common pattern seen in falcons, where species inhabiting the same areas aren't necessarily closely related. The RAG-1 analysis suggested it may be close to the sooty falcon however this seems unlikely due to what we know of the sooty falcons and its relationship to other species (Wink & Ristow, 2000). It may be that the New Zealand falcon has some relationship with the main kestrel group evidenced by its position just outside it in the Cyt-b analysis. However, the data are insufficient to draw a strong conclusion on the relationship.

### **2.4.2 Structure of the *Falco* genus:**

I found at least two monophyletic groups in accordance with Helbig (1994) who also found evidence that at least two monophyletic groups occur- the *Heirolfalco* subgenus and the typical kestrels. The term heirolfalco is commonly used for a complex of so-called desert falcons (subgenus-*Heirolfalco*) (Cade 1982; Tucker and Heath 1994; Eastham 2000). The subgenus *Heirolfalco* was defined by Kleinschmidt (1901) as a group of ecologically and morphologically similar species that replace each other in



different parts of the world. They share similarities in body proportions (broad-based long wings, long tails) and have a soft plumage structure, which are assumed to have evolved convergently for similar hunting modes in dry open environments. Currently five species are ascribed to the *Heirolfalco* subgenus: the lanner falcon (*F. biarmicus*), the saker falcon (*F. cherrug*), the gyr falcon (*F. rusticolus*), the black falcon (*F. subniger*) and the laggar falcon (*F. jugger*). These birds all live in open 'desert' areas of Europe, Asia, Africa, Australia and America. Typically they capture their prey close to the ground (Nittenger et al, 2005). It seems to be a contentious issue as to whether or not peregrines also belong in this group, with some research keeping them apart in their own group (Wink et al, 2004). Peregrine falcons are also large falcons. Phylogenetic reconstructions suggest that the large falcons are a recently diverged group, and this is supported by fossil records (Sibley and Ahlquist 1990, Helbig et al 1994, Nittenger et al 2007). Molecular data have placed the emergence of peregrine Falcons in the late Pliocene or early Pleistocene (Wilson et al 1987, Tarr and Fleischer 1993). Peregrine falcons are thought to be closely allied to prairie falcons (*F. mexicanus*) on the basis of molecular and behavioural evidence (White et al. 2002), (The prairie falcon sits just outside the main *Heirolfalco*/peregrine group in our analysis). My analysis suggests that while the peregrines fall into their own species complex (*pelegrinoides*, *peregrinus* and other possible subspecies) they are also closely allied with the subgenus *Heirolfalco*. It has been suggested that the black falcon also belongs in the heirofalcon group based on Cyt-b sequences (Wink et al, 2004). My data supports this; the black falcon was closely placed within the *Heirolfalco* subgenus in both the RAG-1 and Cyt-b analyses. The grey falcon (*F. hypoleucus*) and prairie falcon are also placed close to the heirofalcon group. My results suggest that either the *Heirolfalco* genus could either include the lanner, laggar, gyr, black and saker falcons or extend to contain eight species (saker, gyr, lanner, laggar, peregrine, black, grey and prairie falcons).

Within the literature, there is some ambiguity in the relationship between the saker and gyr falcon. Discussions have been on-going for a long time as to how they relate to one another, specifically, whether they are in fact the same species but adapted to different environments. A recent paper has discovered it is possible to correctly place a

bird into its species group (saker or gyr) via microsatellite analysis (Dawnay et al, 2007) suggesting they are in fact distinct species. It is likely, however, that they may hybridise in the wild where the ranges overlap (Nittenger et al, 2007).

My results do not fit with the current species groupings of the kestrels. The kestrels were named due to similarities in appearance and behaviour, described as small, long-winged, long-tailed, short-toed falcons (Groombridge, 2002). The kestrel group is assumed to contain 13 species (Village, 1990), whose global distribution comprises a single New World species (the American kestrel) and 12 Old World species (Boyce and White, 1987). Generally, they are split into 'typical' and 'aberrant' kestrels. My data suggests the structure of this group maybe slightly different than previously thought. My analysis includes the common kestrel (*F. tinnunculus*), Mauritius kestrel (*F. punctatus*), Seychelles kestrel (*F. araea*), banded kestrel (*F. zoniventris*), nankeen kestrel (*F. cenchroides*), lesser kestrel (*F. naumanni*), Malagasy kestrel (*F. newtoni*) and greater kestrel (*F. rupicoloides*) together. The American kestrel (*F. Sparverius*) does not cluster with this group of kestrels but sits alone, in a more basal position. Wink (2004) recognised this split also, and suggested it may form a clade with the aplomado falcon however my analysis did not support this. My analysis also suggests the banded kestrel (*F. zoniventris*) fits in this group (not in 'aberrant' kestrels). It appears the 'aberrant' kestrels mentioned earlier do not exist as a group.

The Malagasy kestrel and Seychelles kestrel are sister species which makes sense as they inhabit the islands of Madagascar and the Seychelles respectively. They appear to be closer to the common kestrel than to any other African species (however we are missing samples of two African kestrels in the analysis). My analysis does not support a close relationship to the Mauritius kestrel as suggested by Groombridge (2004). The evolutionary relationship between African kestrels and their radiation across the Indian Ocean islands has attracted much debate from evolutionary biologists. The molecular phylogeny supports an Old World origin for the kestrel group, and indicates a recent radiation of kestrels from Madagascar towards Mauritius and

the Seychelles (Groombridge et al, 2004). Future analysis may support a conclusion on this topic.

The remaining *Falco* species are harder to group. The sooty and Eleonora's falcon are thought to be sister species. Wink (2004) concluded they had similarities in ecology and behaviour and thought they may be related to hobbies. My Cyt-b analysis supports this, with the sooty falcon, Eleonora's falcon, Eurasian Hobby (*F. subbuteo*), little falcon/Hobby (*F. longipennis*) and African hobby (*F. curvierii*) forming a clade. This clade could be called a hobby group, and could include the orange breasted falcon (*F. dieroleucus*) at the base of the branch. This grouping is supported by Wink (2004), he suggested that the sister species the red footed falcon (*F. vespertinus*) and the amur falcon (*F. amurensis*) may show affinities with this group as well, but they don't fall clearly within this group in my analysis or his. The red necked falcon (*F. chicquera*) seems to fall close to the *Heirofalco* group which indicates that the other common name red necked merlin maybe misleading. The Australian brown falcon (*F. berigora*) has a very unclear placing. Based on feather protein analysis, it is thought to be closely to the New Zealand falcon (Olsen et al, 1989), but this relationship is not supported by my analysis. The placement of the brown falcon, New Zealand falcon, Dickinson's kestrel and the aplomado falcon are somewhat obscure, making it difficult to draw clear conclusions. The Dickinson's kestrel is thought to be close to the banded kestrel (*F. ardosiaceus*) and grey kestrel (*F. alopex*) (Groombridge et al, 2002). We do not have sequence data on this grey kestrel (*F. ardosiaceus*) or the fox kestrel (*F. alopex*) however; the Dickison's kestrel seems close to the aplomado falcon (*F. femoralis*) in my analysis. It is Possible we would see a different pattern if we had data on grey and fox kestrels.

My analysis suggests a lack of biogeographic signal within the *Falco* genus, Griffiths (2004) identified a signal within the family Falconidae but this did not extend to the genus level. Within each clade we see representatives from across the globe and even closely distributed species such as those from within South America don't seem to be closely related. My analysis suggests that within the genus *Falco* there is an abrupt

radiation forming a group of closely related species, resulting in the unresolved polytomy's present in the RAG-1 analysis. This close relationship creates difficulties in identifying the relationships between species and hence any possible biogeographic signal present.

### **2.4.3 Gene resolution:**

I found a pronounced difference in the variation, and therefore resolution with the two genes used in this analysis. While we used a similar fragment length for both genes, RAG-1 showed a lot less variation in the alignment than Cyt-b. This supports the idea that the falcon genus has undergone a shallow radiation and rapid diversification. It also suggests a difference in the two genes resolution. Because mitochondrial sequences evolve faster than nuclear sequences, the Cyt-b data set has a greater than 3-fold advantage over RAG-1 in the proportion of variable sites and an almost six-fold advantage in the proportion of informative sites (Griffiths et al, 2007). However, while RAG-1 has very few informative characters they are of very high quality (Griffiths et al, 2007).

Griffiths et al (2007) examined the efficacy of Cyt-b and RAG-1 in a study of the phylogeny of Falconidae. It was found that while RAG-1 was successful in resolving relationships among the different genera it was not sufficient to resolve species relationships within the genus *Falco*. However, there are also some saturation problems with Cyt-b with evidence of multiple substitutions and further problems with anomalies in base composition (Griffiths et al, 2004). RAG-1 has been used in resolving the phylogeny of the accipiteridae, successfully determining relationships within the family (Griffiths et al, 2007). It seems probable that RAG-1 is good for wider resolution in orders and families, but not so good for relationships at the species level, where Cyt-b is more informative.

This begs the question whether the groupings we see in our analysis represent the true relationships within the *Falco* genus or whether they are a result of unsatisfactory gene resolution. While I recognize the inconsistencies in the genes and the analytical problems mentioned above, both genes used have been successful in determining relationships in other bird taxa (Griffiths et al, 2007). This gives confidence my analysis accurately represents the true history of the *Falco* genus.

#### **2.4.4 Conclusion:**

My analysis suggests a rapid diversification in the *Falco* genus that has formed a closely related group of species. The New Zealand falcon is part of this genus and may sit closest to the kestrel group. The New Zealand falcon is not sister to any other single species from either Australia or South America, even though it may be similar ecologically. There was a clear difference in the variation between the Cyt-b and RAG-1 genes used, but this can be explained by the difference in evolutionary rates of mitochondrial and nuclear DNA. This may have some influence on the inconsistencies in our results however, I can be confident that our analysis represents an accurate history of the genus *Falco*.

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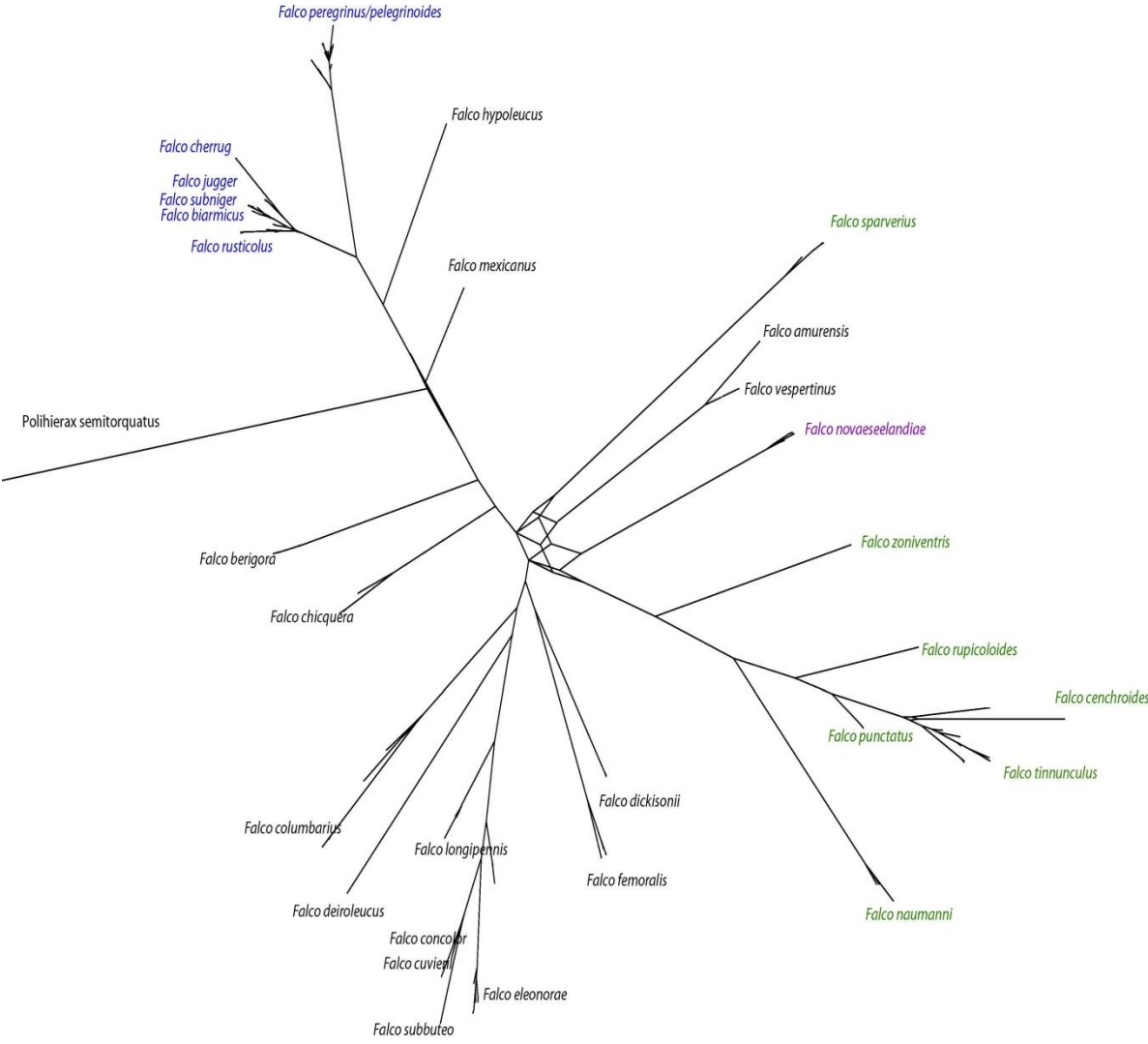
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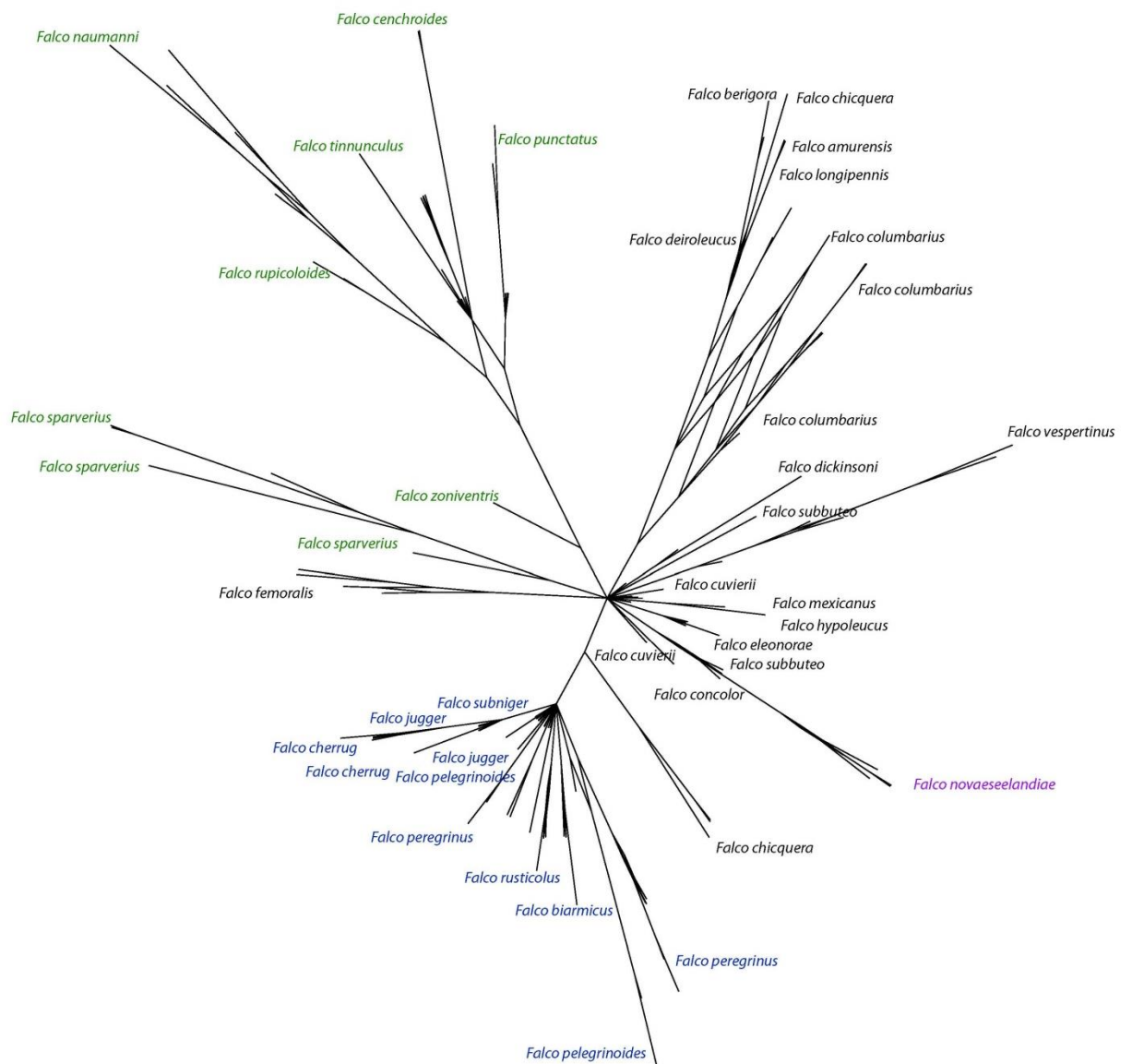
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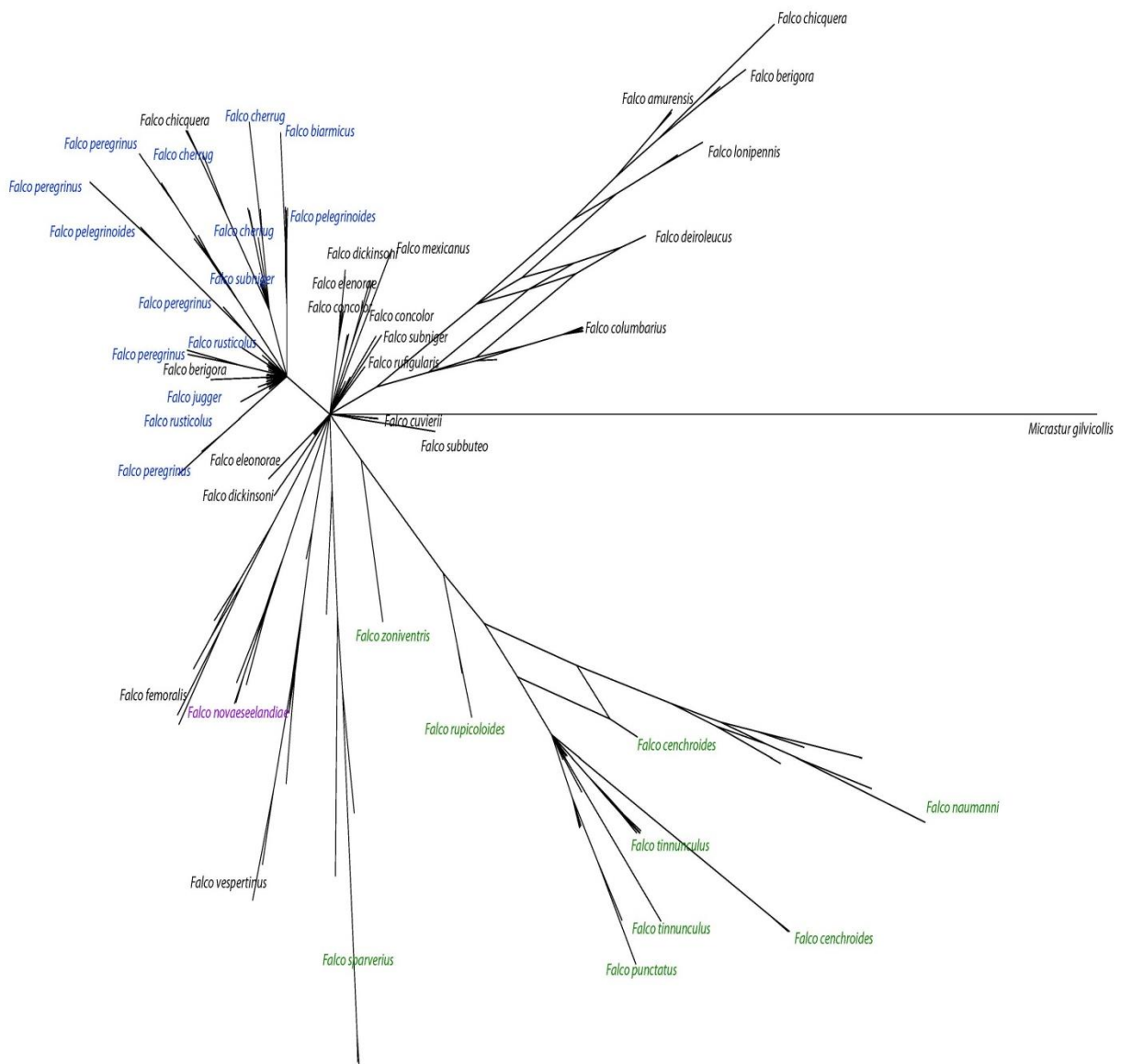
# Appendix:



Appendix 1: Bayesian analysis network using Cyt-b data (n=31), tree constructed using Splitstree software using the out group *Poliheirax semitorquatus*. The New Zealand falcon (purple text), kestrel group (green text) and the *Heirofalco* subgenus (blue text).



Appendix 2: Bayesian analysis using RAG-1 sequences, trees constructed with Splitstree software (n=31). No out group was used in this analysis. The New Zealand falcon is in purple text, the *Heirofalco* subgenus is in blue text and the kestrel group in green text.



Appendix 3: Bayesian analysis network using RAG-1 sequence data and with *Micrastur gilvicolis* as the out group (n=31). Shows *Heirofalco* subgenus (blue text) and kestrel group (green text). New Zealand falcon is seen in the purple text.

## Chapter 3:

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### **Polymorphism in the New Zealand falcon.**



Adult male New Zealand falcon, Benmore, Marlborough (Photo- Lena Olley).

## 3.1 Introduction:

### 3.1.1 New Zealand bird life:

One of the reasons New Zealand intrigues evolutionary biologists is that it displays biological characteristics of both oceanic islands and continental land masses (Goldberg et al, 2011). New Zealand has faced intense climatic and geological activity, much of it in a relatively short time frame; it is not surprising that phylogeographic studies of New Zealand's fauna have suggested that Pleistocene climate cycling had an important influence on the distribution and divergence of several taxa (Trewick & Bland, 2011). The geophysical characteristics and history of New Zealand have resulted in a distinctive avifauna comprising a mixture of old and young lineages. Several New Zealand bird species exhibit sub species or morphic differences, and a frequent occurrence within species and closely related groups is that South Island individuals tend to be larger than North Island individuals. Bird species that have been recorded as displaying this pattern of size variation include kaka (*Nestor meridionalis*), saddleback (*Philesturnus carunculatus*), kea (*Nestor notabilis*) and robins (*Petroica australis*). Examination of fossil bones shows that several recently extinct species such as the laughing owl (*Sceloglaux albifacies*), adzebills (*Aptornis otidiformis*, *Aptornis defossor*), owl night-jar (*Aegotheles novaezeelandiae*) and the extinct wrens (family: Acanthisittidae) also displayed this inter-island size difference.

Morphometrics of the laughing owl long bones (humeri, femora, tibiotarsi and tarsometatarsi) reveal that North Island individuals were significantly smaller than those in the South Island (Gill, 1996). There was however only a small sample size from the North Island, making it difficult to test the spatial pattern of this phenomenon. Data for the New Zealand robin appears to indicate a clear size difference between North and South Island individuals, and recent genetic evidence supports the idea of their being two subspecies reflecting this difference (Miller & Lambert, 2006).

This general pattern among New Zealand birds appears to fit Bergmann's rule, however contrary examples exist in some species including the takahe. The South Island takahe (*Porphyrio hochstetteri*) and extinct North Island takahe (*Porphyrio mantelli*) showed a different size and shape between the two islands (Trewick, 1996). The North Island takahe was taller than the South Island takahe but not much heavier, and they also had different sized bills (with the North Island birds having smaller bills) (Trewick, 1996). However, the classification of distinct morphs or subspecies based on morphology alone may be erroneous, and are often not supported by genetic data. For example the current morphological and ecological data on population structure for the kaka (*Nestor meridionalis*) are ambiguous. There are currently two recognized subspecies, North Island (*N. meridionalis septentrionalis*) and South Island (*N. meridionalis meridionalis*), which are distinguished by plumage and size (Sainsbury et al, 2006). These morphological traits, however, are as variable within islands as between (Sainsbury et al, 2006). Microsatellite analysis shows little population structure in kaka among substantial genetic variation across the country, supporting the idea of extensive gene flow between populations (Sainsbury et al, 2006).

Bergmann's rule states that within a broadly distributed taxonomic clade or lineage, populations and or species of larger size are found in colder environments and those of smaller size are found in warmer regions (Bergmann, 1847). This may occur among populations within a species and between species within a genus. Bergmann's Rule is closely associated with latitude, with a latitudinal gradient in size being typical (Hamilton, 1961). This pattern may occur even with panmixis (complete gene flow) throughout the population, because the trait can be phenotypically plastic and responsive to environmental variables correlated with latitude. In New Zealand Bergmann's rule predicts increasing size from the top of the North Island to the bottom of the South Island.

### 3.1.2 The *Falco* genus:

Polymorphism is the variation within a species independent of sex and ontogeny (Weins, 1999) and usually results in two or more distinct and genetically determined colour morphs in one interbreeding population (Huxley, 1955). Many bird species exhibit morphic variation, with birds of prey in particular include a high proportion of variable species. Of the 237 species of birds of prey, 30% show some degree of polymorphism (Fowlie & Kruger, 2003). The most likely evolutionary correlate of polymorphism in both owls and other birds of prey is population size; different plumage morphs might simply arise in larger populations because of the higher number of mutations (Fowlie & Kruger, 2003).

The niche variation hypothesis is also used to explain polymorphism and examines the difference between specialist and generalist species of predatory birds. It states that the transition from monomorphic to polymorphic plumage is associated with a broad ecological niche (Galeotti & Rubolini, 2004). It is suggested that polymorphic species of raptor are widely distributed and adapted to a broader range of environmental conditions compared to their monomorphic counterparts, resulting in different morphs occupying different parts of a broad niche.

Falcons are small to medium sized, compact, long winged, diurnal birds of prey, all of which are included in a single genus, *Falco*. They range in size and variety from the brightly coloured, wind hovering kestrels, some weighing less than 100 grams to the grouse hungry, arctic gyrfalcons, some of which weigh more than two kilograms (Cade, 1982). There have been up to 40 species described depending on taxonomic treatment, but generally 37-39 are accepted. Falcons differ from other birds of prey in a number of characters. They have a tomial tooth on the upper part of the beak with a corresponding notch on the lower beak designed for killing prey by breaking the neck, and long pointed wings with several features designed for fast flight. These characteristics make falcons highly specialised for the aerial hunting and killing of prey.

Differences in the morphology of widespread falcon species are typically resolved by taxonomic splitting into different species and subspecies. Usually groups are based entirely on subjective morphological differences under the assumption that falcons simply change in shape and colour in response to their environment. Understanding the relationship between phenotypic and genotypic data may be particularly informative in studies of avian taxonomy where plumage characteristics have been historically used to designate species and subspecies (Hull et al, 2010). Many of these species and subspecies designations have been based upon phenotypic differences alone and have not been supported by molecular genetic phylogenies, examples include the gyr falcons (*F. rusticolus*) (Johnson et al, 2007), saker falcons (*F. cherrug*) of central Asia (Eastham et al, 2002) and the peregrine falcons (*F. peregrinus*) that has a worldwide distribution and about 75 morphs recognised in the species alone (White et al, 2013).

Peregrine falcons can have wing lengths as small as 84% of the largest wing length within a sex (figure 3.1) (Johansson et al, 1998). This variation has led to the description of at least 19 more or less uniformly recognized geographical races or subspecies. Life history differences vary among peregrine populations with some possessing a migratory lifestyle, and others sedentary as well as differences in diet and habitat type. These differences create different selection pressures. Johansson et al (1998) examined whether the array of differences among peregrines are adaptive or whether they result from other factors such as neutral drift, as might occur with small founder effects or simply phenotypic plasticity. Variation in life histories can be produced through the plastic response of a single genotype to environmental conditions (phenotypic plasticity) or through local adaptation of genotypes to specific sets of environmental conditions (Sears & Angilletta, 2003). Plastic changes in the phenotype may be adaptive or not, but given appropriate genetic variability, adaptive phenotypic responses to the environment are thought to be able to evolve in populations that encounter predictable environmental change (Via et al, 1995). If the differences seen in peregrine populations are adaptive then this raises the question of why there are similarities between populations that have diverse habitats ranging from



moist tropical forests and hot arid deserts to mountains with associated variation in food sources (Johansson et al, 1998). The mechanistic link between the environment and phenotype that is expressed are complex and difficult to discern (Sears & Angilletta, 2003).

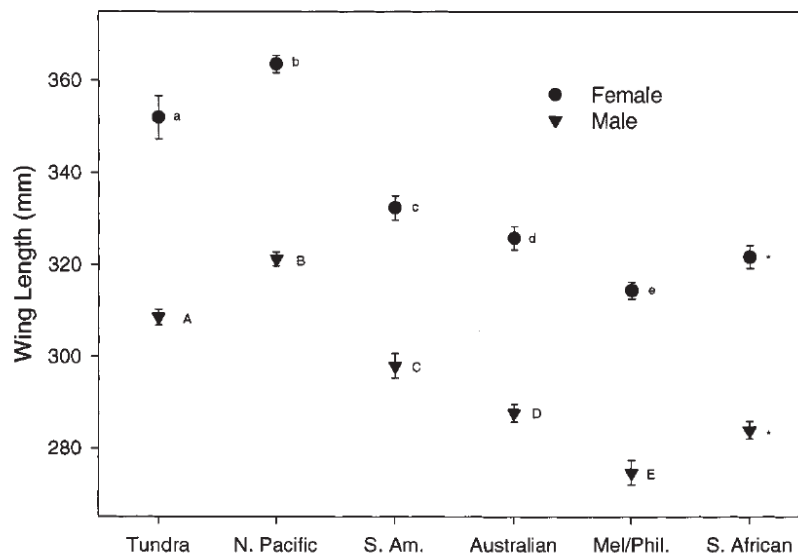


Figure 3.1: Mean wing length for populations of male and female *Falco peregrinus*. Different letters denote significant differences ( $P < 0.05$ ) between means using ANOVA and Tukey's multiple pairwise comparison test (Johansson et al, 1998).

Form and function are expected to be linked (form being a response to environmental factors). This is evident in peregrines where, in hot arid climates of Africa, peregrines have long thin toes compared to their body mass whereas those from colder climates, like the Aleutian Islands, have comparatively thicker and shorter toes relative to body mass. While mainly a food catching function, it is assumed that the foot and tarsus may also have temperature regulating functions, so the variation can be interpreted as being adaptive (White, 1968). Variations in form can also be seen in the wing length, body mass and colour morphs among peregrine falcons around the world. Body mass seems to be consistently positively correlated with latitude following Bergmann's rule suggesting more than phenotypic plasticity.

Absolute bill structure seems to be consistent in the peregrine world over, regardless of location or food type, but there is some evidence of geographical variation when bill

structure is a function of body mass. Other studies suggest variation in the shape and size of the bill may reflect adaptive pressures exerted by diet or at least different dietary niches (Hull 1993, Jenkins 1995). Johansson et al (1998) concluded that there is an 'optimum peregrine' bill size and form whose parameters fall within a narrow range of variation regardless of food spectrum and or whether a diet specialist or generalist. The morphological and geographical variation fixed in peregrine populations attests to the lack of dispersal and thus gene flow among between populations. The striking difference in colour and markings between peregrine falcons in locations as close as Australia and New Guinea supports the inference of low movement and genetic contact between areas (Johansson et al, 1998). While the variation in morphology around falcons is striking, there is still little genetic evidence for the huge number of subspecies proposed (see chapter two, Johnson et al, 2007, Eastham et al, 2002 & White et al, 2013).

The saker falcon and gyr falcon are morphologically similar. Both vary in colour from near black to near white and their geographic ranges overlap in many areas. Typically the gyr falcon is a northern bird with the saker falcon inhabiting areas closer to the Middle East and central Asia (Eastham et al, 2002). It is possible that gyr falcons and saker falcons are sibling species or allopatric populations of the same species (Cade, 1982). However, recent microsatellite analysis has shown evidence for the presence of two species (Dawnay et al, 2008).

Saker falcons also show morphometric variation within the species; however the naming of the subspecies does not seem to reflect the observed morphological variation (Eastham et al, 2002). Although the majority of morphometric variation in saker falcons is within regional populations (among individuals), but some size variation has a geographic basis (Eastham et al, 2002). Saker falcons from the eastern regions (Kazakhstan, south-east Russia, Mongolia and China) are largest, whereas saker falcons from the south-central (Southern Kazakhstan, Turkmenistan, Kyrgyzstan, Uzbekistan and Tajikistan) and north-central regions (north and north-west Kazakhstan) are smaller (Eastham et al, 2002). Although not statistically significant,

western saker falcons (mainly south-west Russia) were on average smaller in size than eastern saker falcons. The wing length of saker falcons was positively correlated with increasing altitude (Eastham et al, 2002) following Bergmann's rule.

### **3.1.3 The New Zealand falcon conundrum:**

The New Zealand falcon is a medium sized falcon with considerable variation in size and colouration (figures 3.2 & 3.3). Males range in size between 37-44 cm in wing length and 252-342 g in weight, while females usually range between 44-47 cm in wing length and 420-594 g in weight (Hoyo et al, 1994). However, on occasion females over 700g have been recorded from the Otago region. Males generally weigh 56-62 % of the female body weight. The New Zealand falcon is generally dark in colour with a variable number of white bars present on the wing and tail. They are lighter on the underside with darker streaks; while the thighs are rufous with dark streaks. Juveniles are darker in colour on the underside than the adults (Cade, 1982). The fleshy parts around the eye and cere, as well as the legs, are grey blue in juveniles and bright yellow in adults (Cade, 1982). We know that New Zealand falcons are an adaptable species (evidence includes the ability to inhabit and breed in exotic pine plantations) and that they are widely spread throughout New Zealand. According to a number of hypotheses (for example the niche variation hypothesis (Galeotti & Rubolini, 2004) these attributes make the New Zealand falcon a prime species in which polymorphism may occur.

Three morphs of the New Zealand falcon are generally recognised (Fox, 1977), (see chapter one). To some extent morphology, ecology and geographic range appear to be correlated but to date no genetic work testing this has been conducted. Fox's (1977) proposal for the three morphs of New Zealand falcon was as follows:

Bush falcon- North Island and the North West South Island.

Eastern falcon- Eastern South Island from Marlborough to the bottom of the South Island and across to the mid-west coast.

Southern falcon- Fiordland, Stewart Island and the Auckland Islands.

Bush falcons are reported as being smaller and darker than the Eastern falcon and the Southern falcon is said to be intermediate (Fox, 1977). In light of the variation seen in other falcon species it is not surprising that the New Zealand falcon also shows such variation. Discussion over the New Zealand falcon's taxonomy has been going on for many years, since at least 1773 (chapter one). The proposal of three New Zealand morphs has now become generally accepted in New Zealand. The Department Of Conservation now includes the three morphs separately in the threat classification document (Robertson et al, 2013) and most New Zealand bird books quote three morphs (Scofield & Stephenson 2013, Heather & Robertson 1996, Watola 2009) Even the International Union for Conservation of Nature (IUCN) records the three forms (<http://www.iucnredlist.org/>). However, Fox (1988) urged that pending further research New Zealand falcons should be treated as a single variable species.

There are a number of possibilities as to how the size variation in the New Zealand falcon may be partitioned. There may be three size morphs corresponding to the current morph proposal or there may be a pattern similar to other New Zealand birds with a North and South Island size variant. There may also be a gradual change corresponding to a latitudinal gradient reflecting Bergmann's rule.

### **3.1.4 Aims:**

This research aims to reassess evidence for morphological variation in New Zealand falcons and to identify how this variation is distributed across the landscape. Specifically, I examine the evidence for three distinct morphs, in contrast to the alternative hypothesis of a gradient in size consistent with Bergmann's rule.



Figure 3.2: The typical female 'Bush' falcon from Galatea (central North Island) (top) is much darker and smaller than the "Eastern falcon" from Otago (bottom). Photo courtesy of Andrew Thomas (Wingspan Birds of Prey Trust).



Figure 3.3: A typical male 'Bush' falcon from Wellington (top) is much darker and smaller than the 'Eastern' falcon from Queenstown (bottom). Photo courtesy of Andrew Thomas (Wingspan Birds of Prey Trust).

## **3.2 Methods:**

### **3.2.1 Sample Collection:**

Morphometric data was compiled from various sources around the country (figure 3.4). Raw data was extracted from Fox (1977), and supplemented this with data from research undertaken in Kaingaroa forest during 2000 and 2011 and measurements from captive and rehabilitated birds (Wingspan Birds of Prey Trust). Dead falcons handed in to Department of Conservation area offices and skins from three museum collections (Tepapa, Canterbury and Auckland) provided novel data. Data was compiled on six different linear dimensions -wing length, tail length, tarsus length, centre toe length, bill length as well as overall weight/mass. As data was retrieved from several sources not all metrics were obtained from every specimen. The most abundant measurement recorded was wing length which is thought to be the most reliable measurement for predicting overall body size (Grant, 1971). Weight is highly variable among individuals, at different times of the day and among seasons. Therefore, wing length was the primary measurement considered in the analysis. As the age of the bird will have an impact on wing length only adult birds were used in the analysis.

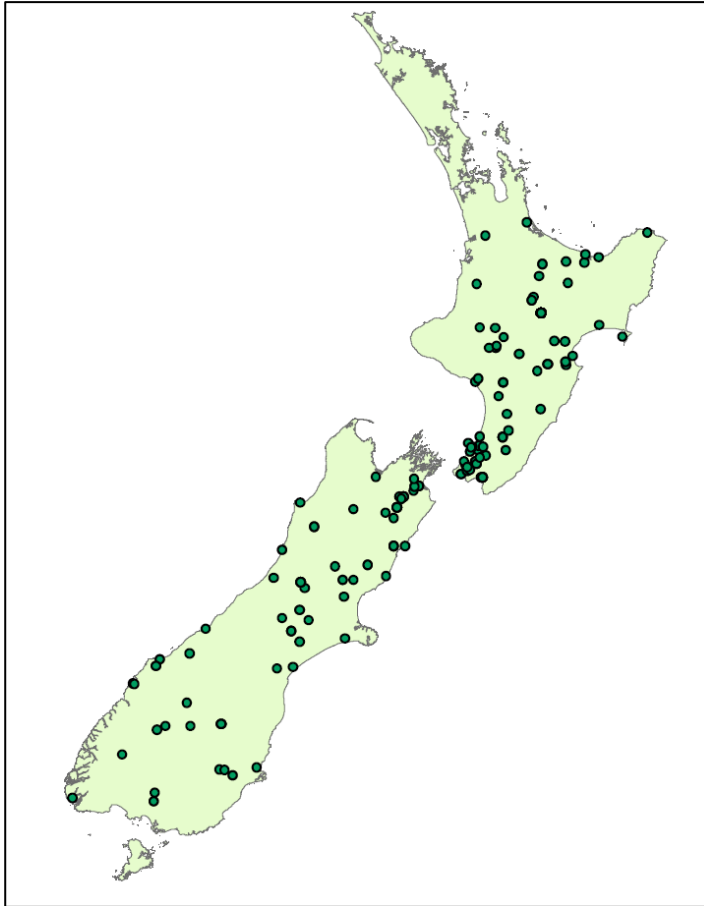


Figure 3.4: Sampling locations of New Zealand falcons used in this study (n=229). Some locations yielded multiple individuals from a single GPS point, for example there are 38 individuals from the Kaingaroa forest. Samples from the Auckland Islands are not shown.

### 3.2.2 Measurements:

*Wing length*- Wing length is defined as the distance on the closed wing from the foremost extremity of the carpus to the tip of the longest feather. The bird wing is not a simple structure, because as well as the lateral curvature of the primaries there is also a camber across of the wing. Because of this there are three ways of measuring the wing length of a bird depending on the extent to which the wing is flattened (Spencer, 1965). Measuring the un-flattened wing gives the minimum length, a flattened chord gives an intermediate length and flattening and straightening gives the maximum length. According to the Ringers Manual (Spencer, 1965), using the flattened and straightened chord is the most accepted way to measure a wing and it is

this morphometric that is used in the International Ringers Exchange, as it seems to give the most repeatable and accurate results (Spencer, 1965). To measure the straightened and flattened chord you slide a stopped rule under the folded wing and the carpal joint positioned against the stop. Gentle pressure on the median or greater coverts, flattens the wing against the ruler removing the camber both a long and across the wing. The longest primary feather is then straightened along the ruler from the base to the tip.

*Tail length-* Tail length is measured from the skin between the deck (central) feathers to the tip of the longest feather (Spencer, 1965).

*Tarsus length-* Tarsus length is the length of the tarsometatarsal bone (Spencer, 1965). This is measured from the notch of the intertarsal joint and the other at the lower edge of the last complete scale before the toes diverge, or, when the foot is bent downwards to 90 degrees to the tarsus, from the notch to the point that it bends.

*Weight-* Weight is measured by putting the falcon in a cloth bag hung from a spring measure with a scale of suitable range (up to 1000 grams).

*Culmen to tip-* Culmen length is measured in three ways; from the bill tip to the cere, to the feathering or to the skull. The most common method for raptors is from the tip of the bill to the cere. To measure this, the callipers should be lined up against the tip of the bill and then closed until the callipers meet the base of the cere (Spencer, 1965).

*Center toe-* Center toe is measured as the length of the middle toe from the joint with the tarsometatarsus (the joint becomes visible when the toe is bent slightly downward) to the posterior end of the middle toe not including the claw. To measure this, the toe is bent downward and gently stretched to allow the measurement to be taken on the dorsal side.

*Sources of variation and error in measurements:*

When measuring wing length there may be some variation depending on the amount of pressure applied in the flattening process. However, through using the flattened



wing chord measurement most inaccuracies caused by curvature should be eliminated. It is also important to keep the wing naturally folded (Spencer, 1965).

Both tail and wing length measurements are subject to time of year and the moult cycle, to mitigate this, birds in moult were not included in the analysis. Weight may vary with crop content and season, although this was not controlled for, size differences may still be significant despite this.

Fox (1977) investigated whether or not live bird wing measurements and museum specimens (dry skins) could be comparable. He dissected wings of dried museum specimens and found that the carpometacarpus and primary feather were unlikely to change during the preparation of a specimen skin. As well as this he found that the contour of the wing is unlikely to change. He suggested that the loss of fluid at the carpal joint could create at maximum 1mm of shrinkage (Fox, 1977).

### **3.2.3 Analysis:**

#### *Summary Statistics:*

All morphological analyses were completed with males and females separated due to the reverse sexual dimorphism present in falcons. To understand how the morphometric measurements related to each other SAS (v 9.4) was used to identify correlations among each of the measurements. Means and coefficient of variation were calculated for each metric. Wing length was selected as the primary data because it is representative of full body size and was obtained for the largest number of individuals. Wing lengths were plotted in a frequency histogram to visualise the distribution of lengths, and therefore bird size, throughout the samples. Outliers were removed from the analysis.

#### *Cluster Analysis:*

Cluster analysis was used to determine if there were any patterns in wing lengths within New Zealand. Each individual bird was placed into a New Zealand region based

on its location, 14 regions were identified for females and 16 regions for males as the sampling for males came from more locations than females. Without attributing them to any type (Bush, Eastern or Southern), Ward's Minimum Variance Cluster Analysis (SAS (v 9.4)) was used to construct dendrograms.

Mclust (v4.3) package (Fraley et al, 2014) in R (i386 3.0.2) was used to examine wing lengths and tail lengths. The results were displayed in a scatterplot of wing lengths versus tail lengths. Mclust (v4.3) is a Model based approach and assumes a variety of data models and applies maximum likelihood estimation and Bayes criteria to identify the most likely model and number of clusters (Fraley et al, 2012). Specifically, the Mclust function in the Mclust package selects the optimal model according to Bayesian Information Criterion (BIC) for EM (algorithm for parameterized Gaussian mixture models) initialized by hierarchical clustering for parameterized Gaussian mixture models. One then chooses the model and number of clusters with the largest BIC (Fraley et al, 2012).

#### *Habitat and Latitude Analysis:*

Several methods were used to explore relationships between falcon morphometrics and habitat, latitude and location. To test for support of Bergmann's rule significant latitude was compared to wing length. Latitude data was obtained using New Zealand topographic maps online. The relationship between latitude and wing length was graphed using Kaleidagraph (v 4.5).

To test for correspondence of the proposed regional boundaries and morphotypes the habitat in which each individual bird lived in was correlated with its wing length using GIS software. Each individual's location (latitude and longitude) was entered into ARC Map (ESRI, 2010) to estimate the primary habitat it most likely lived in. As records of sampling locations were sometimes poorly defined and falcons typically forage over a wide area, a buffer of 20 km<sup>2</sup> around each mapped location was used. Primary habitats fell into eight categories according to the LENZ database- High producing exotic grassland, Low producing exotic grassland, Exotic forest, Indigenous forest, Tall tussock

grassland, River, Lake and pond and Gravel and rock. For most locations, the primary habitat covered more than 80% of the 20km<sup>2</sup> area. Once habitats were recorded for each bird sampled, bar charts were constructed in Sigmaplot (v 12.5) against mean wing length for each habitat.

ANOVA (Tukeys test) in SPSS (IBM SPSS Statistics 20) was used to test for significant differences in mean wing length among habitat types. The habitat and latitude data for each sample were also included in the Cluster Analysis/linear modelling using R (i386 3.0.2).

#### *Hierarchical Analysis:*

In order to determine the most important factor affecting the population structure data was compiled in a Hierarchical analysis. Based on the results from the cluster analysis and the habitat and latitude analysis, a linear model (R (i386 3.0.2)) was used to perform a Hierarchical analysis to compare the influence of latitude and habitat on the size variation within the morphometric data.

#### *Multi-metric Analysis:*

All analyses were performed separately for wing length and tail length as the dominant variables. However, to test the combined effect of the morphometrics some of the analyses were repeated to include the three metrics; wing length, tail length and tarsus length together. Center toe length and culmen to tip length were not as abundant as tarsus length so were not included. A principal component analysis (IBM SPSS statistics 20) and the Mclust (v4.3) analysis were performed using all three morphometrics.

#### *Distribution of wing lengths:*

A Hartigans' dip test (v 0.75-5) for unimodality (Maechler, 2014) in R (i386 3.0.2) (based on 4000 replicates) was used to determine if the frequency of wing lengths in the North and South island had a bimodal or a normal distribution. Outliers were

removed. Significant differences between mean wing length for males and females were tested for using a T-test (IBM SPSS Statistics 20).

### 3.3 Results:

#### 3.3.1 Summary Statistics:

A total of 121 female and 123 male falcons were sampled. Male wing lengths varied from 123mm to 268mm while female wing lengths ranged from 212mm to 308mm (table 3.1). The distribution of wing lengths appears to display a bimodal distribution (figures 3.5 and 3.6). Wing and tail length were positively correlated in both males and females. Weight was also positively correlated with wing length in females and tail length in males (tables 3.2 and 3.3). Center toe length and weight were positively correlated with tarsus length in both males and females while culmen to tip or bill length was correlated with weight, wing length and center toe length in females but not in males. The variation in weight is quite large between regions as well as individuals (figure 3.7), the largest birds are from the South Island, specifically Otago, the West coast and Canterbury.

Table 3.1: Total number, mean, standard deviation, coefficient of variation and minimum and maximum values of each metric variable in male and female New Zealand falcons.

Male	Variable	N	Mean	Std Dev	CV	Minimum	Maximum
	weight	60	257.22	40.27468	12.71306	170	372
	wing length	110	240.5636	20.36424	7.703651	123	268
	tail length	109	168.1853	9.02891	3.711452	147	190
	tarsus	109	56.36606	2.45848	3.697355	50	62.5
	center toe	51	40.91569	3.28088	7.903323	36	49.5
	culmen tip	24	17.8125	1.04312	5.720396	14.8	20.3
Female	Variable	N	Mean	Std Dev	CV	Minimum	Maximum
	weight	79	488.7646	97.27369	14.63397	245	846
	wing length	103	280.534	16.07691	3.695288	212	308
	tail length	98	192.1939	15.88597	6.239398	108	234
	tarsus	102	63.46961	4.05169	5.390497	54	74
	center toe	45	48.56	4.86614	6.90517	41.6	64
	culmen tip	34	22.17647	1.44664	5.239173	18.5	27.2

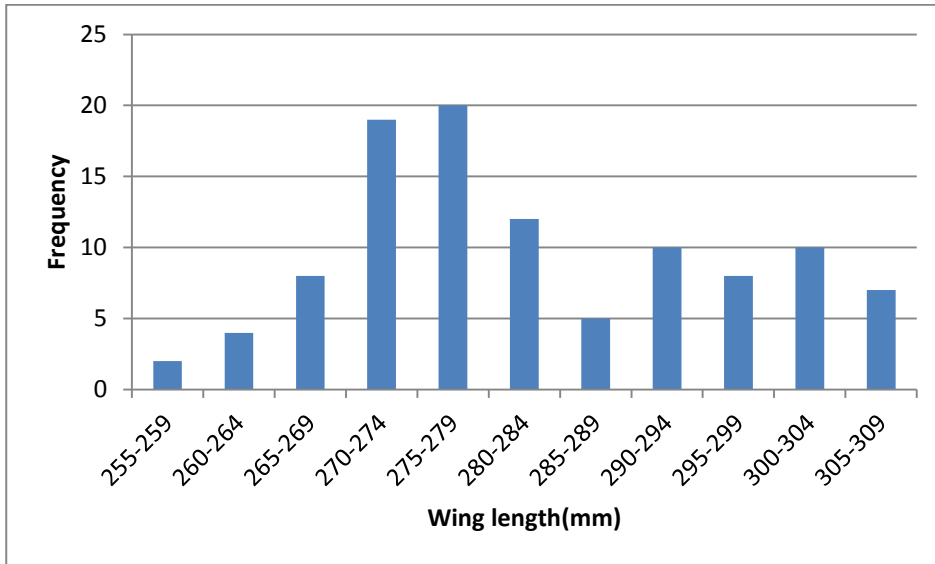


Figure 3.5: Distribution of wing lengths (mm) in female falcons.

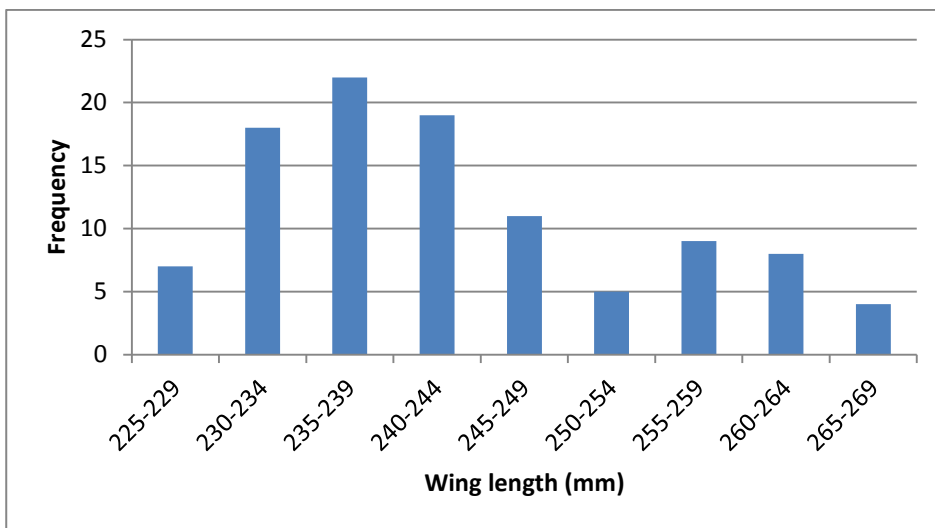


Figure 3.6: Distribution of wing lengths (mm) in male falcons.

Table 3.2: Correlation of body measurements in male New Zealand falcons (n=110). Pearson's correlation coefficient, p-value and sample size are given. Significant correlations ( $p < 0.01$ ) are in bold.

Pearsons Correlation Coefficient	Weight	Wing length	Tail length	Tarsus	Center toe	Culmen tip
Weight	1					
n=	60					
Wing length	0.28207	1				
p-value	0.0335					
n=	57	110				
Tail length	0.48223	0.37349	1			
p-value	<b>0.0001</b>	<b>&lt;.0001</b>				
n=	57	106	109			
Tarsus	0.11299	0.05668	-0.09118	1		
p-value	0.3942	0.5677	0.355			
n=	59	104	105	109		
Center toe	-0.78008	-0.04061	-0.21054	0.3853	1	
p-value	<b>0.0028</b>	0.7795	0.1381	<b>0.0063</b>		
n=	12	50	51	49	51	
Culmen tip	0.05887	-0.00086	0.20857	0.1278	0.77418	1
p-value	0.7999	0.997	0.3516	0.5611	0.0143	
n=	21	22	22	23	9	24

Table 3.3: Correlation of body measurements of female New Zealand falcons (n=103). Pearson's correlation coefficient, p-value and sample size are given. Significant correlations ( $p < 0.01$ ) are in bold.

Pearsons Correlation Coefficient	Weight	Wing length	Tail length	Tarsus	Center toe	Culmen tip
Weight	1					
n=	79					
Wing length	0.56553	1				
p-value	<b>&lt;.0001</b>					
n=	72	103				
Tail length	-0.02573	0.54114	1			
p-value	0.8375	<b>&lt;.0001</b>				
n=	66	94	98			
Tarsus	0.29958	0.20841	0.01005	1		
p-value	<b>0.009</b>	0.0405	0.9243			
n=	75	97	92	102		
Centertoe	0.62264	0.17338	0.25427	0.74053	1	
p-value	<b>0.0058</b>	0.2662	0.1042	<b>&lt;.0001</b>		
n=	18	43	42	39	45	
Culmentip	0.63522	0.47233	0.47877	-0.09473	0.85414	1
p-value	<b>&lt;.0001</b>	<b>0.0063</b>	0.0179	0.5941	<b>0.0017</b>	
n=	32	32	24	34	10	34

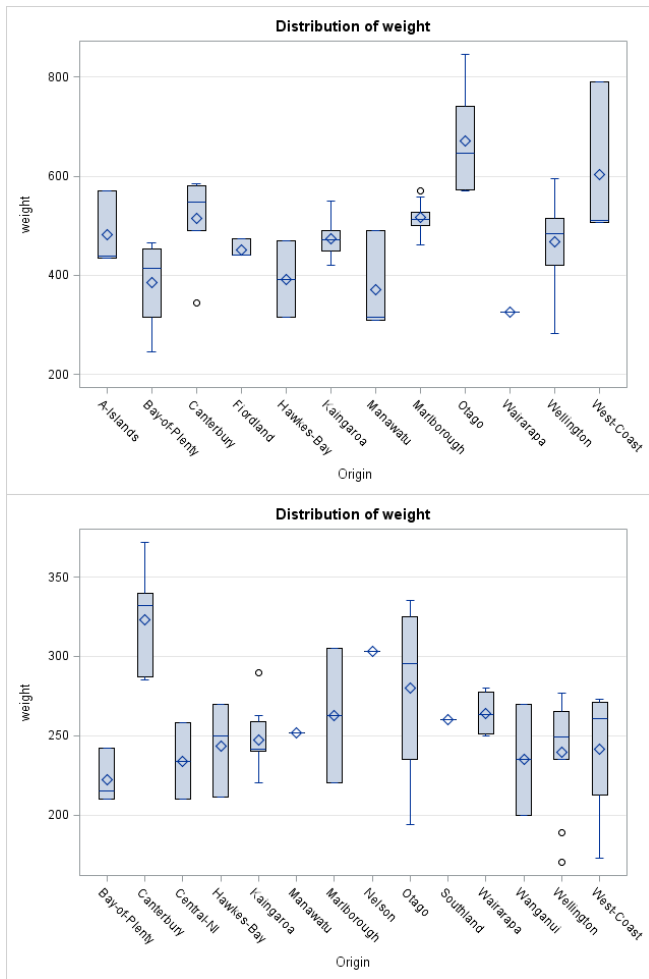


Figure 3.7: Variation in New Zealand falcon weight among regions. Females, right (n=79) and males, left (n=60).

### 3.3.2 Cluster Analysis:

Cluster analysis produced in SAS (v.9.4) shows clear groupings of falcon wing lengths for the North and South Island birds (figures 3.8 and 3.9). The exceptions being that among female falcons, individuals from the Auckland Islands and Fiordland cluster with the North Island. Males, however, show a clear North Island/South Island split. Female falcons from South Island regions had a wing length above 278mm, while North Island birds were all below 277 mm. Males tended to show more overlap in wing lengths, with the South island birds falling between 235-259mm while North Island birds were between 213-240mm (table 3.4). This North/South split is supported by the Mclust (v4) cluster analysis, which also shows there are two clusters in the wing and tail length data, corresponding to the North Island and South Island (figure 3.10).

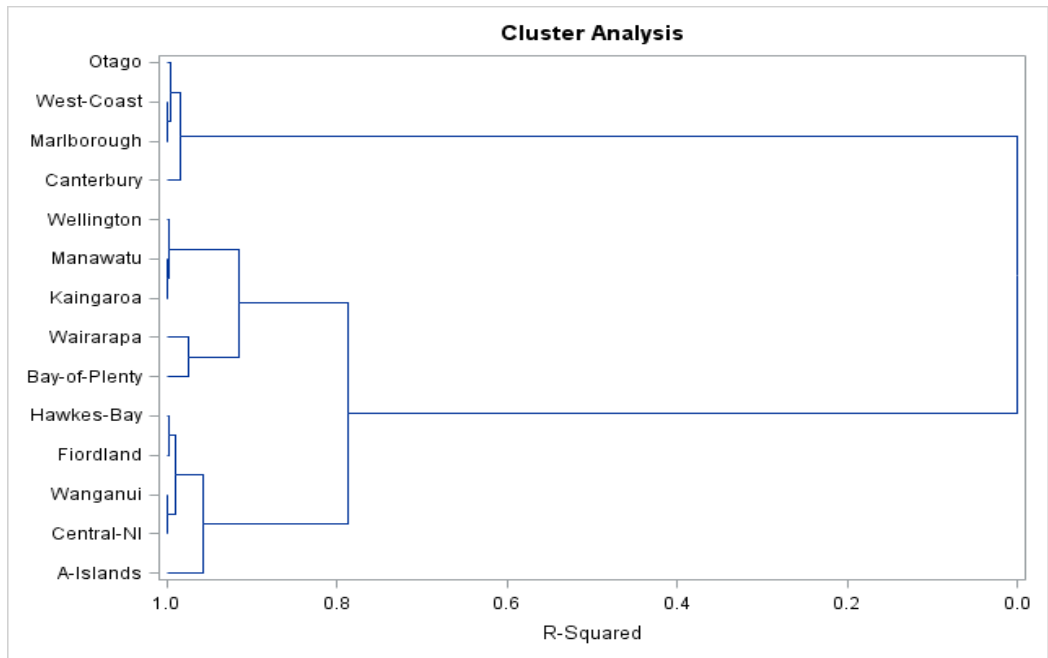


Figure 3.8: Dendrogram of mean wing lengths of female New Zealand falcons (n=103) from 14 regions of New Zealand.

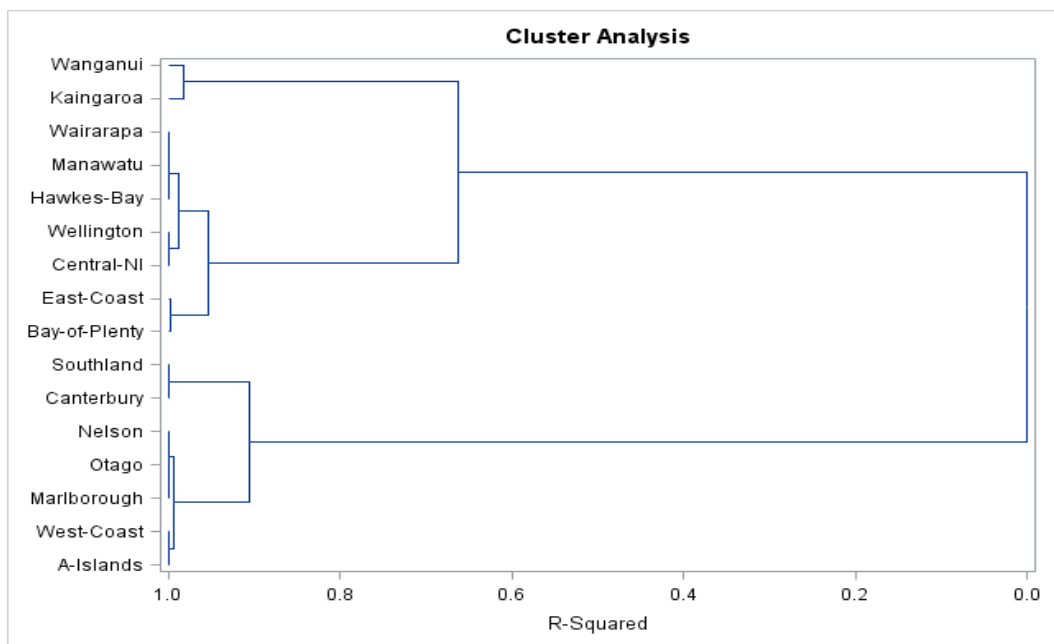


Figure 3.9: Dendrogram of mean wing length of male New Zealand falcons (n=110) from 16 regions in New Zealand.



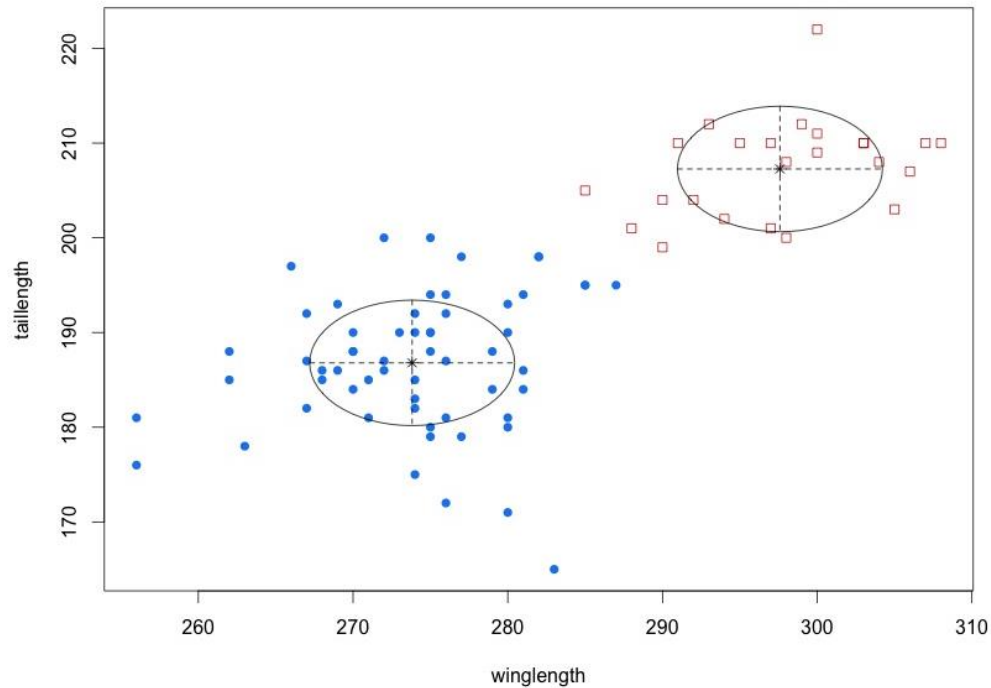


Figure 3.10: Relationships between wing length and tail length of female New Zealand falcons (n=94) obtained using Mclust (v.4). Ellipses indicate two clusters (blue and red) representing North and South Island individuals.

### 3.3.3 Habitat and Latitude Analysis:

Results from the habitat analysis suggest that falcons with the longest wing lengths came from areas of open country. In both males and females the largest birds came from locations dominated by 'Tall tussock grassland' and 'Low producing grassland' (figures 3.11 and 3.12). These two habitat types were mostly located in the South Island.

The smallest wing lengths were those in 'indigenous forest'. ANOVA (Tukeys test) showed significant differences in wing length between some of these habitats. In males 'Indigenous forest' and 'Low producing exotic grassland' had significantly different mean wing lengths as did 'high producing exotic grassland' and 'low producing exotic grassland'. Females had fewer habitat types recorded but significant differences can still be seen between 'Indigenous forest' and 'Low producing grassland'.

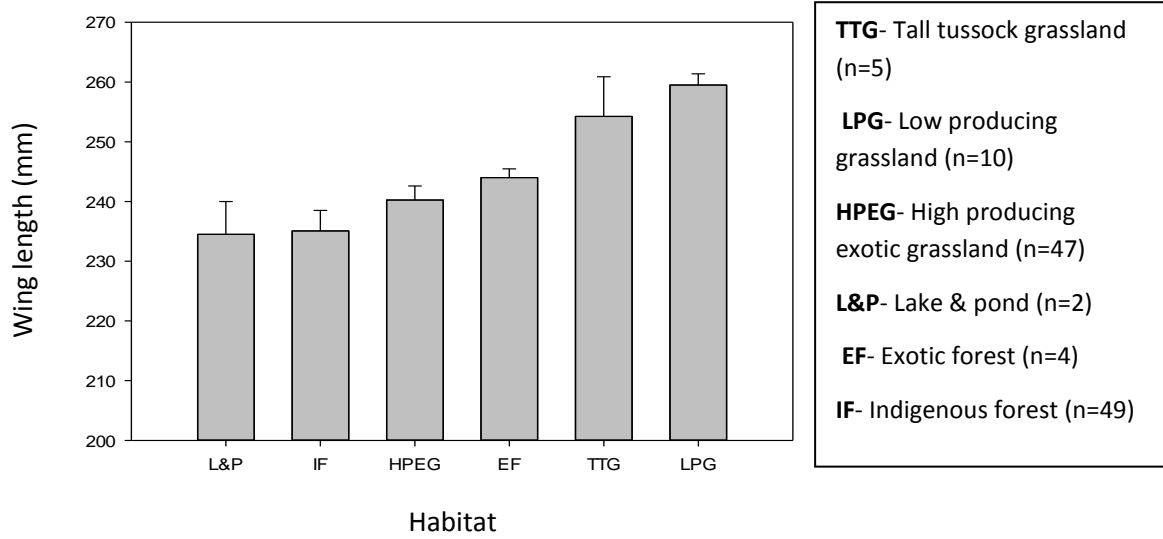


Figure 3.11: Average wing length (mm) of male New Zealand falcons (n=117) versus primary habitat type for individual falcon localities. Tukeys test identified significant differences between IF/LPG (p=0.000) and HPEG/LPG (p=0.010).

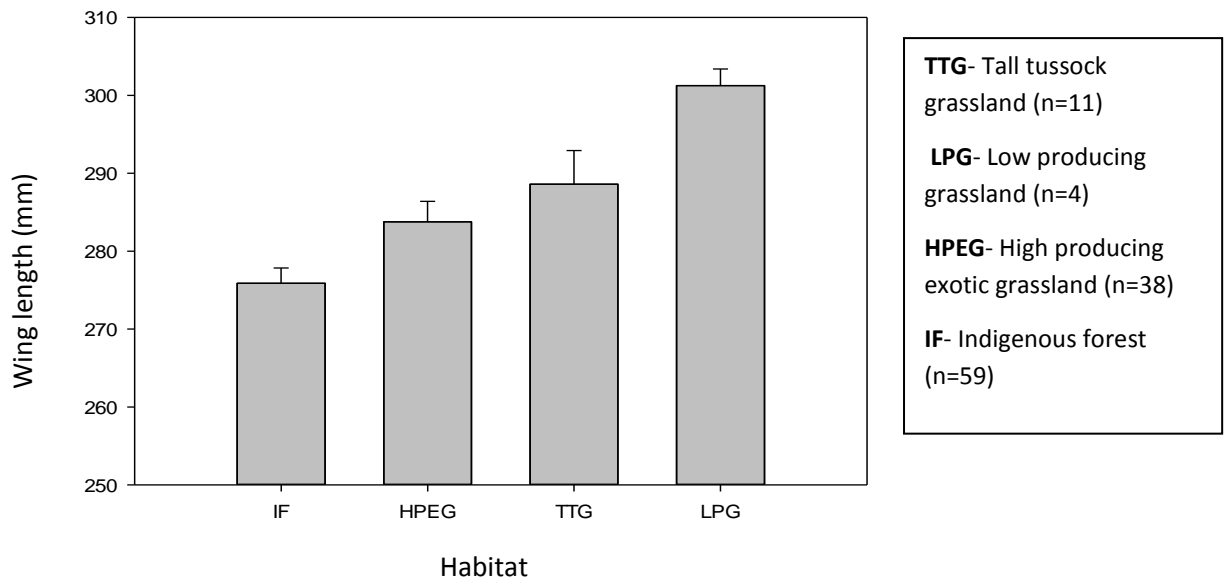


Figure 3.12: Average wing length (mm) of female falcons (n=112) against primary habitat types for individual localities. Tukeys test identified a significant difference between IF and LPG (p=0.007).

There was a significant positive correlation between wing length and latitude (figure 3.13), however, individuals from the Auckland Islands did not fit this pattern and appear to be intermediate in size. When data for falcon wing lengths for the North and South Islands samples were analysed separately they reveal different slope intercepts. This results in a step like transition between the North and the South Island rather than an even gradient as expected if assuming Bergmann's rule (figure 3.14). Very little effect of latitude was apparent within islands, for either the North or South Islands. The R-values are low when the islands are analysed separately signalling minimum slope in the regression lines. If the North and South Island data are combined, the low-latitude North Island falcons have short wings and the high latitude South Island falcons have longer wings. Thus, the latitudinal gradient is an artefact of pooling data from two distinct size distributions. That is not because there is a North-South cline, but rather a North Island / South Island discontinuity. Mean wing length for male falcons in the North Island is 233.86 mm while South Island birds had a mean wing length of 250.2 mm. North Island females had a mean wing length of 270.53 mm and South Island females had 292.33 mm (table 3.4).

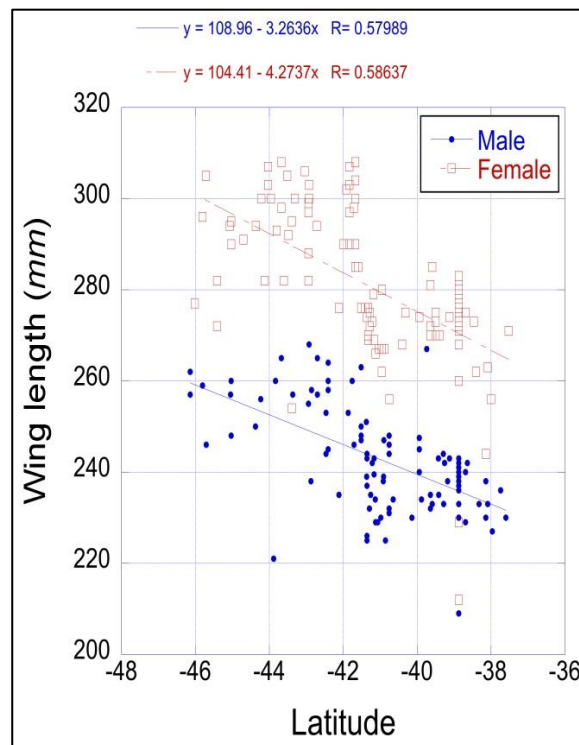


Figure 3.13: Relationship between latitude and wing length (mm) in male and female falcons. The Auckland Islands were excluded. (Females=red, males=blue).

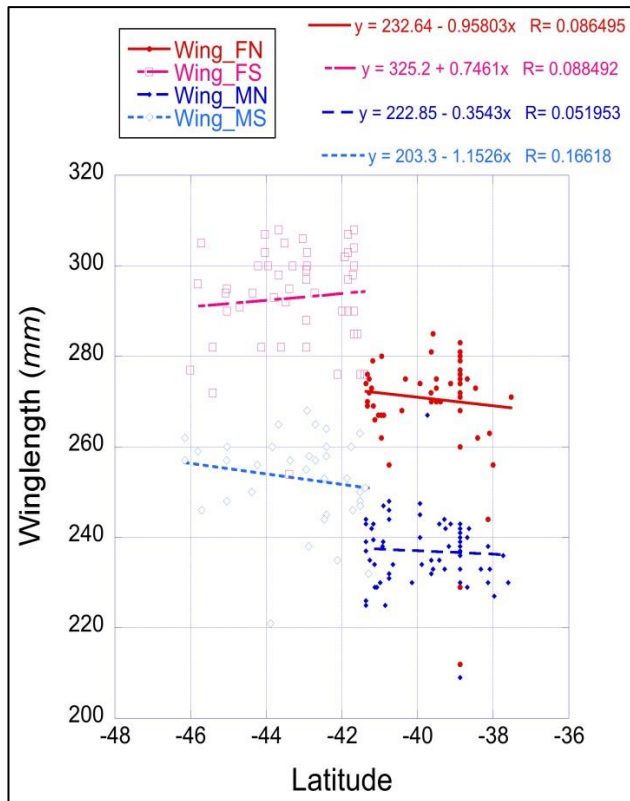


Figure 3.14: Relationship between latitude and wing length (mm) in male and female falcons. The Auckland Islands were excluded. (Females - red/pink, males in blue, north island=closed circles, south island=open squares). Regression line included for each of the islands.

Table 3.4: Sample sizes, Mean wing lengths (mm), Standard deviation and Standard Error of female (top) and male (bottom) New Zealand falcons in North and South Islands.

	north/south	N	Mean	Std. Deviation	Std. Error Mean
Wing length	north	60	270.53	11.857	1.531
	south	52	292.33	11.197	1.553

	north/south	N	Mean	Std. Deviation	Std. Error Mean
Wing length	north	71	233.86	19.731	2.342
	south	46	250.20	16.132	2.379

### 3.3.4 Hierarchical Analysis:

In order to determine which factor (latitude, habitat, north/south split) maybe most important in the structuring identified in this analysis a Hierarchical analysis was used. It was discovered that there is a significant influence of the North and South Island on wing length. Latitude is a significant factor when analysed alone as is the habitat the bird comes from, but when added together with the North/South factor, these become far less important suggesting that they encompass some of the variation partitioned between North and South Islands (Table 3.5).

Table 3.5: Hierarchical analysis of each factor affecting wing length of New Zealand falcons (Habitat code, Latitude and the North/South Island split).

Coefficients	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	252.230	34.0867	7.400	3.52e-11 ***
Habitat code	0.9121	0.8124	1.123	0.264
North/South	22.1272	4.1515	5.330	5.66e-07 ***
Latitude	0.1332	0.9483	0.140	0.889

### 3.3.5 Multi-metric Analysis:

When adding other morphometric measurements other than wing length and tail length (which are positively correlated) to the analysis, we see a different pattern. A principal component analysis (SPSS) performed on the data including wing length, tail length and tarsus length suggests something slightly different to the analysis using only wing length and tail length. Two clusters are identified with Kaingaroa forest individuals in one cluster and the rest of the population in another cluster (figure 3.15). Cluster analysis (Mclust v 4) with all three metrics shows different pattern again with three clusters identified a North and South Island split and also a cluster consisting of the Kaingaroa birds (figure 3.16).

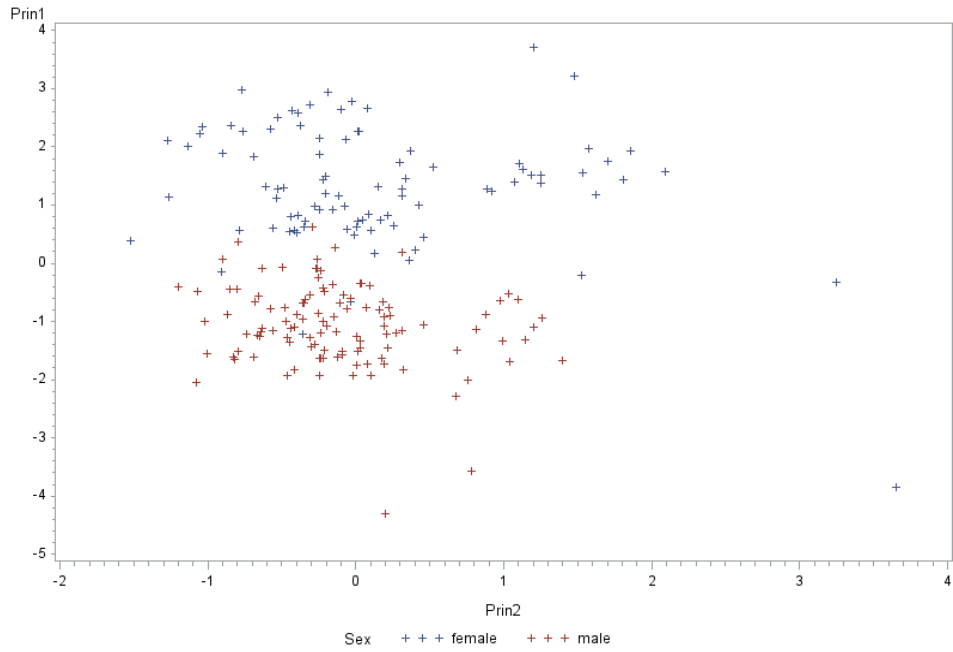


Figure 3.15: Principal Component Analysis of wing length, tail length and tarsus length in male and female falcons (blue=females, red=male).

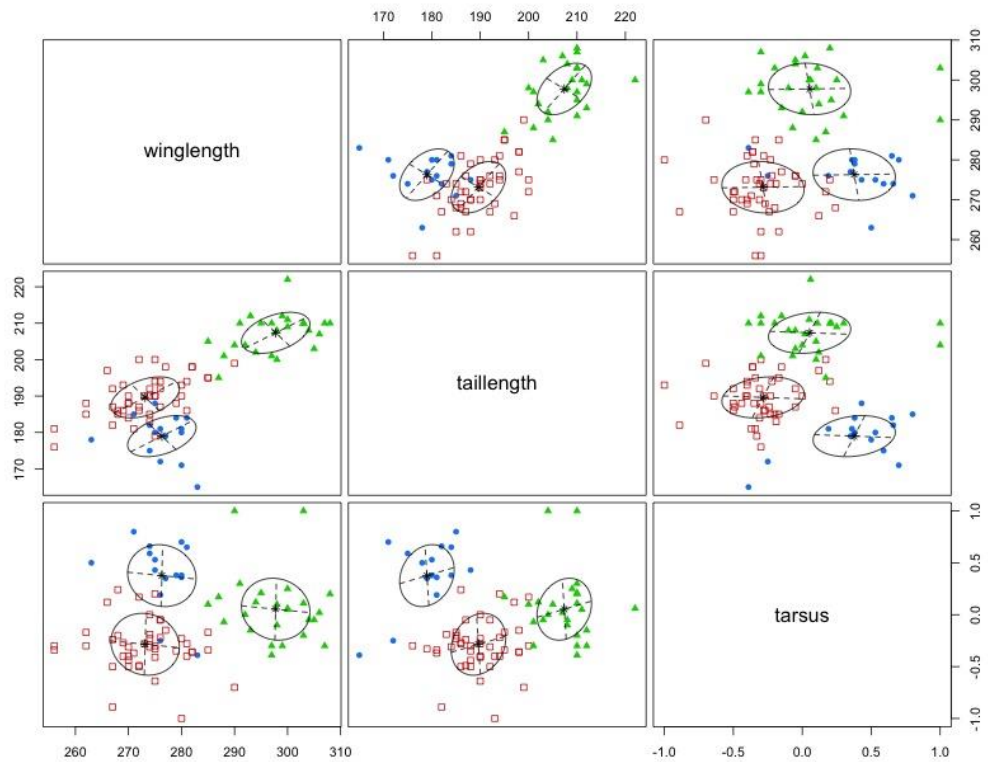


Figure 3.16: Mclust (v4) analysis in R of female falcon wing length, tail length and tarsus length. Colours representing three clusters- Red= South Island individuals, Green= North Island and Blue= Kaingaroa individuals.

### 3.3.6 Distribution of wing lengths:

Frequency histograms show how the size distribution in wing length is attributed between the North and South Islands (figures 3.17 and 3.18). The pattern seen suggests a bimodal distribution. This was tested for using Hartigan's dip test for unimodality (based on 4000 replicates). This test suggested that there wasn't a unimodal distribution but there wasn't significant evidence for the distribution being bimodal.

$D = 0.0909$ ,  $p = 0.5232$  (males)

$D = 0.1061$ ,  $p = 0.4627$  (females)

Mean wing lengths of female and males falcons are significantly different between the North and South Islands ( $p = 0.000$ ).

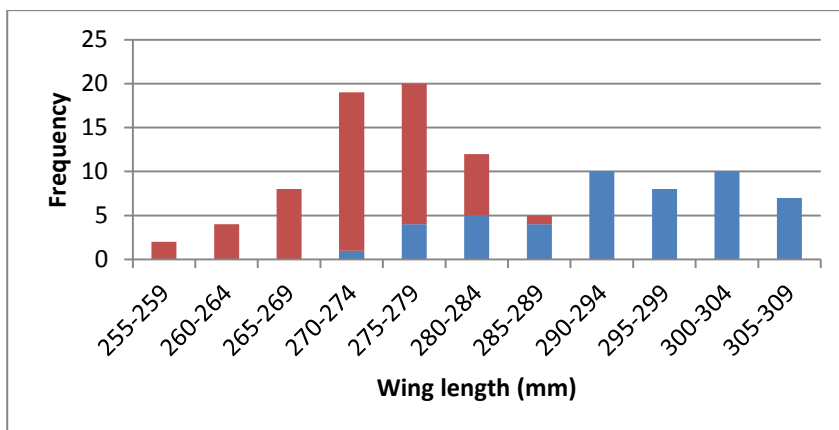


Figure 3.17: Distribution of wing length frequencies in female falcons in the North (red) and South (blue) Islands.

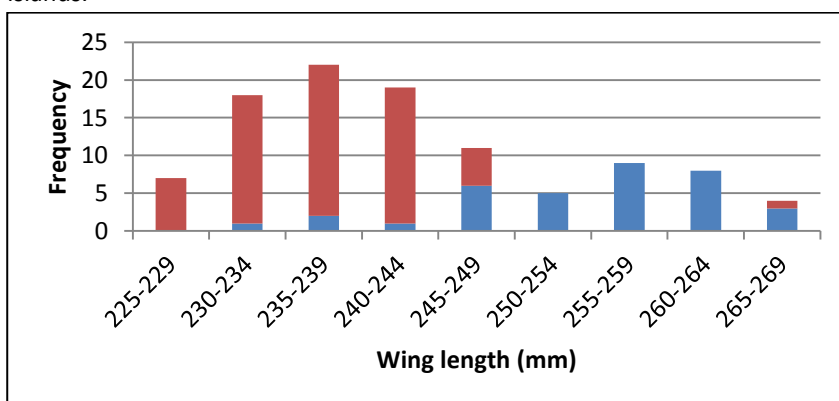


Figure 3.18: Distribution of wing length frequencies in male falcons in the North (red) and South (blue) Islands.

## **3.4 Discussion:**

### **3.4.1 The North- South split:**

The analysis suggests that there is little support for the occurrence of three morphs of New Zealand falcon (Bush, Eastern and Southern) as originally proposed. Instead, there is evidence of a clear distinction in size between the North and South Islands, and while there is some evidence of a gradual change corresponding to latitude this appears to be minimal and maybe an artefact of sampling. Mean wing lengths are significantly longer in both male and females in the South Island than they are in the North Island. Wing length is reported to be a good indicator of true body size (Grant, 1971) suggesting this pattern may be true of overall body size as well. A study of merlins (*Falco columbarius*) examining the best indicator of overall body size showed the highest correlations were obtained for body length and wing length. Either one of these characters seems to provide a fairly good estimate of overall body size (Wiklund, 1996). Fat-free body weight is the most satisfactory measure of body size, but can also be very sensitive to age, body condition, sex, crop content at time of weigh and season. This analysis suggests that there was a difference in weight between some regions, however variation among individuals was large and no further analysis on this was carried out.

Tarsus length appears to be the poorest indicator of overall body size. Fox (1977) found no significant difference in tarsus length among the three New Zealand falcon morphs he proposed; however with a small sample size it is difficult to get significance in such a small measurement (Fox, 1977). It maybe that tarsus length is too hard to measure accurately or is under a different selection pressures than wing and tail length. A variety of bird species on the Tres Marias Island group show a significant difference in tarsi length but not in wing length suggesting that wing length (and body size) and tarsus lengths do not vary concordantly among specimens from a given locality (Grant, 1971). This analysis showed no correlation between tarsus and wing length. It seems it is possible for selection to act upon body size and tarsus length at



least partly independently, so it is reasonable to suppose that large variation in tarsus length is not simply a consequence of large variation in body size. In light of this and the results from Fox (1977) it may be that tarsus length is not reliable as a measure of regional species differences.

When tarsus length was included in the analysis, a slightly different impression of population structure resulted. The cluster analysis with wing, tail and tarsus length recognized the north and south clusters but it also split the North Island birds and the birds located in Kaingaroa forest. There could be many possible reasons for this; it is possible we are seeing a true difference in size at least in wing length between Kaingaroa falcons and those found throughout the rest of the country or it could be a bias in the sampling or human error in the measurements taken.

The suggestion that the falcons in Kaingaroa forest are a different size to all others seems unlikely but Kaingaroa forest does have a high level of research being undertaken on falcons so it is possible that dense sampling at this site has influenced the distribution of sizes obtained. New Zealand falcons are known to inhabit exotic pine forest throughout the entire country so there is no clear reason why Kaingaroa forest alone should influence bird shape or size. There is little research on falcon population numbers throughout New Zealand so it is difficult to know if Kaingaroa forest supports a larger population than other areas. The samples in this analysis were taken by three separate researchers introducing the probability of sampling error. In light of this uncertainty it cannot be conclusively determined that Kaingaroa forest falcons represent a distinct group.

### **3.4.2 Habitat, movement and latitudinal effects:**

There is a clear gradient of shorter to a longer wing in New Zealand falcons as the latitude decreases throughout New Zealand. This gradient however, becomes less obvious when the New Zealand land mass is considered as two different islands. The

analysis found little support for Bergmann's rule because within each island there was little evidence of a gradient in wing length. So while latitude may have some effect it appears to be minimal. It is also important to point out that there are many different selection pressures influencing body size in addition to that of adaptation to external temperatures (Mayr 1956). Factors such as food availability (McAdam and Boutin 2003) and population density (Damuth 1981) are known to lead to changes in body size-related traits (such as body mass or tarsus length) in many species (Husby et al, 2011).

Falcons are capable of moving great distances; some species are migratory and can routinely travel long distances. However, most falcon species appear to display highly philopatric behaviour, and they choose to remain and/or return to a nesting territory where they remain for most of their lives. This behaviour is likely to have an impact on the movements and distribution of a species and therefore the population structure. Peregrines are a worldwide species yet they have failed to colonise a number of suitable regions including New Zealand. The species' general tendency of limited colonization of islands is demonstrated by the fact that peregrines took over 90 years to colonize the Krakatau Island archipelago, via Anak Krakatau, after a volcano erupted and sterilized the island group in 1883 (Rawlinson and Zann, 1992). This is despite the fact that peregrines are known to breed on islands throughout Indonesia, including adjacent Sumatra and Java on either side of Krakatau, and post-fledgling dispersers or non-breeding adults had abundant opportunities to arrive within a decade or so at Krakatau (Ferguson-Lees and Christie, 2001). It also appears that peregrines have very low genetic diversity on the islands of Fiji (refer to chapter four for more detail) suggesting little genetic exchange among islands from continents (Talbot et al, 2011).

Sedentary behavior is likely in the New Zealand falcons which have likewise failed to re-colonise areas where they have recently become extinct such as Northland and the Chatham Islands (Aikman & Miskelly, 2004). It seems likely that the ability for long distance dispersal is limited. New Zealand falcons may well travel some distance during natal dispersal but become sedentary after that with little breeding dispersal recorded

(dispersal is discussed in more detail in chapter four). This the lack of movement may explain the size variation we see between the North and South Islands where, juveniles maybe highly mobile over land averaging out sizes within the islands, but not mobile over water. It maybe the Cook Strait is enough of a barrier to prevent birds from crossing between the islands.

Habitat effects on size variation may be even more difficult to understand than latitudinal effects. There is little research on New Zealand falcon movement patterns. A female falcon satellite tracked in Kaingaroa forest was recorded travelling 137 km from her recorded home range. However, she was also recorded as remaining close to her nesting territory for the three years of the study (Holland & McCutcheon, 2007). This makes attributing one individual falcon to one particular habitat in the analysis particularly difficult, when it is possible that they are utilising an array of habitat types at different times or their life. The analysis used 20km<sup>2</sup> buffers around the location from which a falcon originated, but it is highly likely this is not large enough. The exact origin of a number of the samples was also not always known; rather a general area was recorded. Despite this, we still see some evidence of 'open' habitats having individuals with a longer wing compared to the bush habitats which have wing lengths that are shorter. This apparent correlation among habitat and wing lengths may also be explained by the North-South split due as the majority of the 'open' habitats sampled occurred in the South Island and conversely, the North Island had the majority of the covered/bush habitats. There was evidence that particularly large birds occurred in Otago which contains vast open areas of grassland. There have been suggestions that the West Coast of the South Island has birds with shorter wings because the primary habitat is dense bush (Fox, 1977) when in fact this analysis identified quite large birds from West Coast locations.

The correlation of habitat type and size has been examined in wide ranging Red tailed hawks (*Buteo jamaicensis*) in North America (Hull et al, 2008). This hawk has had two subspecies recognized, an eastern and a western form; population structure was examined at a continental as well as at a regional level. Morphologically, hawks from

Texas were much stouter than those from further west. Within western North America population differentiation coincides with the crest of the Sierra Nevada Mountains, suggesting that population structure maybe affected by a habitat break resulting in habitat biased dispersal (Hull et al, 2008). This suggests that population structure of a wide ranging and vagile species may be associated with regional variation in habitat, resulting in different morphotypes.

It may be that juvenile dispersal in New Zealand falcons is in some way influenced by the habitat changes within each of the islands and this may also relate to the habitat individuals may imprint on as juveniles. Peregrine falcons are known to show some philopatric attachment to nest types, with some cliff nesting and some bog nesting. Generally, the peregrine falcon is a cliff-nester the world over, in Finland and parts of northern Sweden, peregrines nest on dry mounds in pool-studded bogs (Nesje et al, 2000). Genetic analysis shows, however, that these populations do not differ significantly suggesting that choice in nest site may depend more on availability than on genetic disposition or origin (Nesje et al, 2000). This supports the idea that the largest barrier in dispersal of philopatric species is likely to be geographical barriers such as water. Beyond this, habitat differences and behaviour such as nest imprinting may play a role in the morphological differences arising in the isolated populations.

### **3.4.3 Phenotypic Plasticity:**

It is believed body mass and dimensions are more plastic than characters such as bill size and which respond to environmental variables in a variety of adaptive ways generally demonstrating directional selection (Johansson et al, 1998). However, the patterns that we see in body mass, such as correlation with latitude, or the remarkable similarities in South American and Australian peregrines populations noted by White et al (1993) must be more than random phenotypic plasticity.

A plastic response of a single genotype to environmental conditions is defined as phenotypic plasticity while an adaptive phenotypic response involves local adaptation of genotypes to specific sets of environmental conditions (Sears & Angilletta, 2003). Reed Buntings (*Emberiza schoeniclus*) are characterised by different bill sizes resulting in two described morphs. Grapputo et al (1998) suggests that a strong selection for large bills in the southern part of the breeding range is probably maintains the geographical differentiation of this species, suggesting an adaptive response. It seems that sufficient time has elapsed for some differentiation and genetic structuring to arise at the four microsatellite loci. But no genetic discrimination was possible with the slower evolving marker (mtDNA sequences), a strong indication that large-billed reed bunting populations probably diverged in relatively recent times (Grapputo et al, 1998).

The existence of two size forms in New Zealand falcons that are correlated with the North and South Island suggests a directional selection, rather than general plasticity and that gene flow is greater within the islands than between them. There are a number of factors that may contribute to an adaptive change in size between the North and South Islands. Temperature, habitat, diet, population density, competition are just a few possibilities. To understand if the size difference between the North and South Islands is an effect of an adaptive response and to examine the extent of gene flow occurring between the two islands a study of neutral genetic markers is needed.

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## Appendix:

Appendix 1: Mean morphometric measurements of female falcons for each region of New Zealand (n=121).

Sex	Location	MEAN					
		Weight (g)	Wing length (mm)	Tail length (mm)	Tarsus (mm)	Center toe (mm)	Culmen to tip (mm)
female	A-Islands	481.1	282.7	191.3	60.3	50	.
female	Bay-of-plenty	384.9	259.2	177.4	61.5	50.4	25.2
female	Canterbury	515.8	298.9	210.1	62.9	48.19	.
female	Central-NI	.	275	190	57.6	44	.
female	Fiordland	451	278.2	198.5	62.2	57	23.8
female	Hawkes-Bay	392.5	276.6	192.6	59.9	45.4	.
female	Kaingarooa	473.1	271.1	178.9	66.5	46.28	21.73
female	Manawatu	371.7	269.9	188.7	61.5	41.6	22.05
female	Marlborough	516.2	294.8	203.8	64.8	48.37	.
female	Otago	670.7	295.4	207.7	65.8	59.57	23.97
female	Wairarapa	325	256	181.5	59.2	.	.
female	Wanganui	.	274	185.5	61.6	45.45	.
female	Wellington	467.7	272.1	188.2	61	47.5	.
female	West-Coast	602.3	296	189.2	65.1	47.9	.

Appendix 2: Mean morphometric measurements of male falcons in each region of New Zealand (n=123)

Sex	Location	MEAN					
		Weight (g)	Wing length (mm)	Tail length (mm)	Tarsus (mm)	Center toe (mm)	Culmen to tip (mm)
male	A-Islands	.	248.78	170.55	56.74	42.03	.
male	Bay-of-plenty	222.33	232.2	167.4	54.28	44.1	18.7
male	Canterbury	322.86	259.46	179.38	56.31	40.7	.
male	Central-NI	234	237.17	164.84	53.72	36.5	.
male	East Coast	.	230	160	55	40.9	.
male	Hawkes-Bay	243.67	240.28	167.5	56.41	41.25	.
male	Kaingarooa	247.25	230.39	157.39	58.6	42.28	17.63
male	Manawatu	252	239.3	167	53.7	37.75	.
male	Marlborough	262.5	250.8	173.4	57.45	41.4	.
male	Nelson	303	252	177	56.6	40.5	.
male	Otago	280.05	235	174.6	58.3	40.4	18.45
male	Southland	260	259.5	180.33	58.2	40.5	.
male	Wairarapa	264.25	240.2	170	55.04	38.65	.
male	Wanganui	235	213	164.75	55.27	37.9	.
male	Wellington	239.7	236.5	164.62	55.38	38.5	17.1
male	West-Coast	241.75	247.5	171.6	56.42	40.8	.

## Chapter 4:

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### **Population Structure in the New Zealand falcon.**



Female New Zealand falcon, Awatere River Mouth, Marlborough (Photo-Lena Olley).

## **4.1 Introduction:**

### **4.1.1 Polymorphism:**

Many falcons are not only sexually dimorphic but vary in size and shape among populations (chapter two); however, many of these described morphs show little genetic differentiation. Intraspecific variation in morphological characters can emerge from several evolutionary forces and lead to extensive sub specific variation (White et al, 2013). Diversification of many traits can occur quickly through processes associated with sexual selection as well as adaptations to local environments, or slowly through the process of geographic isolation and associated genetic drift. Although substantial morphological variation may result from both of these, their influence on neutral genetic variation may be very different (White et al, 2013). When studying intraspecific processes, the importance of determining population genetic structure cannot be overemphasized, the study of which raises many questions with regards to both biological processes and patterns. Analysis of genetic structure can allow alternative predictions to be tested and informs on aspects of life history including aspects such as dispersal, philopatry and mating systems. As a result population genetics is also informative about taxonomy and conservation status (Manel et al, 2003). Detecting genetic discontinuities is necessary for evolutionary biologists and ecologists to understand how the movement of individuals influences the genetic structure of a population. Understanding gene flow is also important for ascertaining the factors that enable or prevent local adaptation (Manel et al, 2003). This is of particular relevance to the New Zealand falcon which is currently taxonomically indeterminate and data deficient in the Department of Conservation's latest threat classification document (Robertson et al, 2013).

Morphological analysis (chapter two) reveals the existence of two morphotypes in the New Zealand falcon, not three as previously thought. The morphs are partitioned between the North and South Islands. The narrow width of Cook Strait means that there is no shift in environmental conditions across the strait greater than the range of

conditions within each of the main islands. This suggests that the difference between the two morphs is not just due to local conditions, gene flow is implicated. The apparent homogenisation of morphotypes within each island is most simply explained by island specific gene flow with the Cook Strait impeding exchange between the islands. This idea predicts that neutral genetic markers (alleles or allele frequencies) will be partitioned between islands. However, if this pattern has established recently the signature might not be visible with available genetic markers.

An alternative hypothesis may be that there is not a clear partitioning of genetic variation. Instead there may be a more gradual change in genetic structure throughout New Zealand. This could be due to isolation by distance, and maybe likely given the traditional view on New Zealand falcon's philopatric behaviour (chapter two).

#### **4.1.2 Genetic variation/structuring in other falcon species:**

The peregrine falcon (*Falco peregrinus*) is one of the most widely distributed terrestrial vertebrates, only humans and associated pest species have wider geographic ranges. This ability of a species to colonize and persist requires plastic and adaptive morphological and behavioural traits and this is apparent in the peregrines large variation in morphology (plumage and to some extent size variation). The distribution in sizes loosely conforms to Bergmann's rule, with individuals being smaller towards the equator. These individuals also show darker plumage patterns. There are as many as 75 described subspecies of peregrine with 19 of these currently recognized, corresponding to different geographic areas (White et al, 2013). This treatment is controversial; the most common question being what degree of difference justifies subspecies classification? Some differences in peregrines are so marked that there are those who would propose species level classification. For example the plumage differences seen in the old world desert forms such as the barbary falcon (*F. peregrinus pelegrinoides*) which some attempt to justify as a distinct species (White et al, 2013).

Despite this morphological variation, there are no deep partitions in the Peregrine falcons Control Region (mtDNA) across their global range (figure 4.1). This is in line with the hypothesis that peregrines have recently diversified. The haplotypes found were shared among populations; this supports the idea that most subspecies statuses are primarily based on morphology. However, subspecies designations as defined by geography account for a significant portion of the genetic variation among groups of peregrines (White et al, 2013). This then poses the question of whether we are best to classify morphs or subspecies based on relatively new genetic data or the plumage diversity and geographical divisions described historically. This question is made more difficult due to recent work examining microsatellites, which show that there may be significant differences between populations of peregrine falcons from different geographic regions (Nesje et al, 2000).

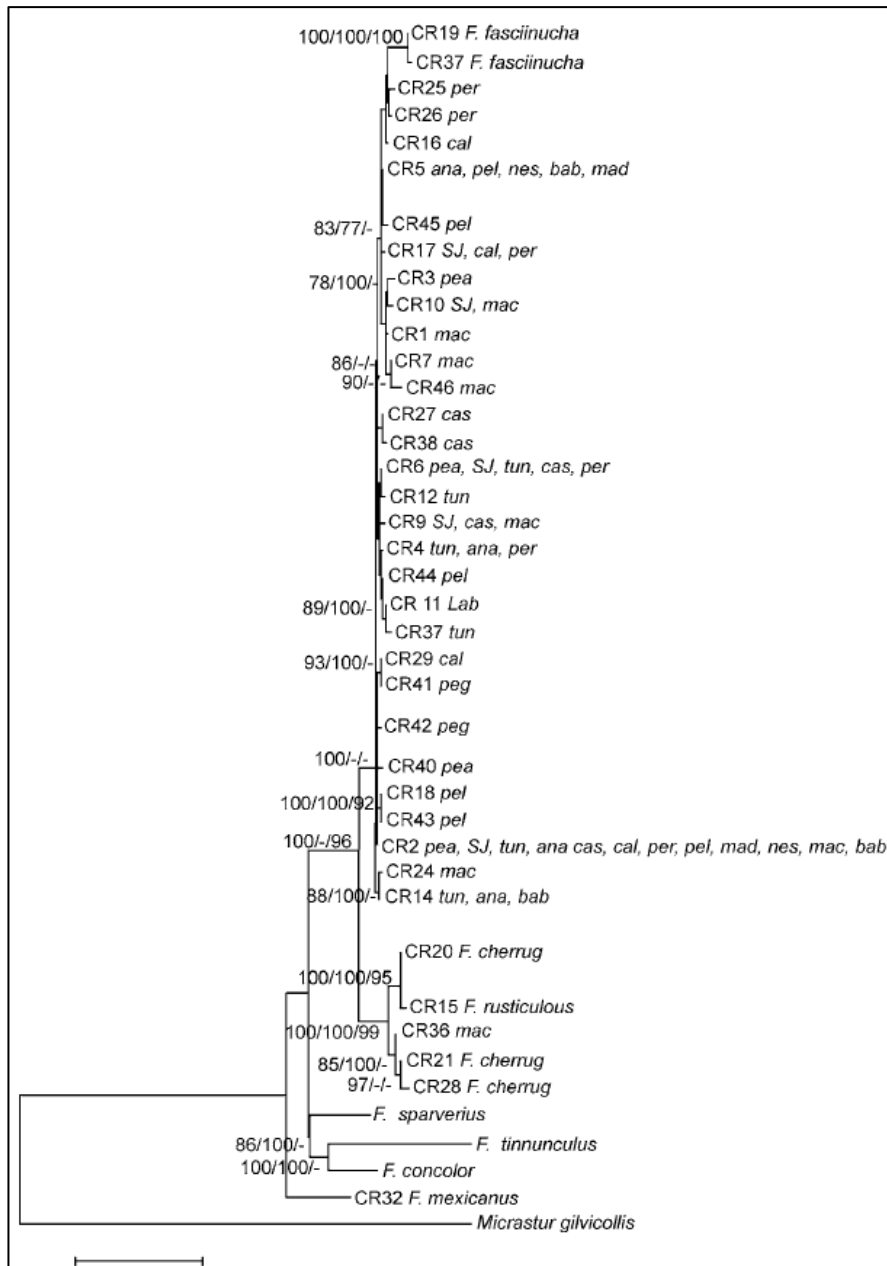


Figure 4.1: Phylogenetic reconstruction of Peregrine falcon (*F. peregrinus*) subspecies based in mtDNA control region. Abbreviations denote subspecies represented by each haplotype: *pea* (*F. p. pealei*), *SJ* (*F. p. San Juan*), *tun* (*F. p. tundrius*), *ana* (*F. p. anatum*), *cas* (*F. p. cassini*), *cal* (*F. p. calidus*), *per* (*F. p. peregrinus*), *pel* (*F. p. pelegrinoides*), *mad* (*F. p. madens*), *nes* (*F. p. nesiotetes*), *mac* (*F. p. macropus*), *bab* (*F. p. babylonicus*), *peg* (*F. p. peregrinator*), and *Lab* (*F. p. Labrador*). Haplotypes are shared among subspecies, with limited nodal support for most clades (White et al, 2013).



Analysis of microsatellite variation among peregrine falcons in the Australian/ Pacific region found the genetic diversity in the Fiji population to be lower than any other island or mainland populations, including Australia, Tasmania and Vanuatu (Talbot et al, 2011). This complete lack of polymorphism at 12 loci has not previously been reported in a natural avian population. However avian taxa often show lower levels of genetic variation on islands than on the mainland. The population was fixed with the most common mitochondrial Control Region haplotype observed in peregrines worldwide (Talbot et al, 2011). By comparison the small bottle-necked populations of the Chatham Island black robin (*Petroica traversi*) and Mauritius kestrel (*Falco punctatus*) show higher levels of polymorphism at fewer loci than Fiji peregrines. The population has an estimated effective breeding population size of 20 pairs, possibly up to 100 individuals (Talbot et al, 2011). Possible reasons behind this lack of polymorphism include geographic barriers such as water as it is an effective barrier to gene flow, meaning the island populations can be highly differentiated. Peregrines are also highly philopatric; they do have a reputation as a 'tramp species' due to their worldwide distribution but dispersers usually return to within 10 kilometers of their hatch site to breed even though they are capable of travelling great distances. Based on this, it is possible that only one or two birds established a population on the islands of Fiji and that very few new birds have migrated there to supplement the population since (Talbot et al, 2011).

A falcon with a large range of occupation similar to that of the peregrine falcon is the gyrfalcon (*Falco rusticolus*) but unlike the peregrine falcon no subspecies have been named. It is however, polytypic and in the past up to 40 subspecies have been proposed but none have been formally adopted (Johnson et al, 2007). These proposed morphs are largely distinguished by plumage differences exhibited between regions. MtDNA analysis revealed little geographic structure but microsatellite analysis showed significant structure among regions and identified separate populations in Iceland and Greenland. Population genetic structure mostly corresponding to geographic areas could then be a response to local environments for camouflage or because of metabolic costs associated with pigment synthesis. The local fixation of the alleles

associated with pigment synthesis would require restricted gene flow between populations however; there is little information on gyr falcon immigration or dispersal patterns on which to base this prediction (Johnson et al, 2007).

The common kestrel (*Falco tinnunculus*) on the Cape Verde islands appears to comprise of two subspecies, possibly three and these have been named on the basis of a number of morphological characters (Hille et al, 2003). Hille et al (2003) used nine microsatellite loci to assess the genetic structure throughout the islands and nearby mainland sites. Between the islands differentiation was strong while the genetic diversity and heterozygosity was lower in island individuals than in individuals from a continental sample in Austria. This suggests limited gene flow between the islands. Hille et al (2003) also found that the genetic distance between island populations was correlated with geographic distance.

These studies are examples of the high level of subspecies classifications within the *Falco* genus, even when a majority of analyses show little genetic support for them. Zink (2004) reports that through analyses of mtDNA sequence data 97% of continentally distributed avian subspecies lack the population genetic structure indicative of distinct evolutionary units. This misinterpretation can be dangerous if it starts misleading conservation management, using resources and funds that could be used elsewhere. Zink (2004) even suggests avian taxonomists should revise classifications by eliminating thousands of subspecies names so that the formal names that remain coincide with known patterns of biodiversity.

#### **4.1.3 Genetic variation/structuring in New Zealand birds:**

New Zealand supports a wide range of other birds which also exhibit morphic differences (chapter two). Quite often morphological variation is partitioned between the North and South Islands. In most cases genetic data have not been used to test the partitioning of the morphological variation. In the New Zealand robin (*Petroica australis*) mtDNA haplotypes were partitioned between the islands (Miller & Lambert,

2006). Size and plumage variation supported the mitochondrial DNA phylogeny in suggesting the North Island and South Island robin clades should be regarded as separate species (Miller & Lambert, 2006) and that these divergences warranted elevation of the two subspecies to full species status. There is less to distinguish the Stewart Island subspecies, in fact, in size and some plumage characteristics it appears more similar to the geographically more distant North Island robin, than the nearby South Island robin. Robins exhibit some life history traits that explain this division, they are known to have limited juvenile dispersal, they are non-migratory and they stay in their breeding territory over winter (Jamieson et al, 2009). These traits are likely to contribute to the partitioning of genetic variation between the two islands, as dispersal of robins across the Cook Strait is likely to be rare.

Kaka parrots (*Nestor meridionalis*) also exhibit morphic variation between the North and South Islands. Currently two subspecies are identified based on plumage and size differences, a North Island and South Island bird (Sainsbury et al, 2006). However, there is little genetic structuring in the species based on a microsatellite analysis of a sample of 126 individuals. The number of alleles and levels of heterozygosity were very similar between the subspecies, and the mean heterozygosity for the entire Kaka assemblage was quite high. Current morphological and ecological information on population structure is ambiguous, with traits being as variable within islands as between. It maybe that kaka have high genetic variation because of a high level of movement (Sainsbury et al, 2006). Compared to the New Zealand robins, kaka are more mobile, little is known on their dispersal but it seems juveniles are likely to disperse reasonably long distances (Moorhouse & Greene, 2002). It appears the dispersal ability of birds is an important factor in the structuring of populations. Adaptive ability may also be a significant factor in population structuring; the niche adaptation hypothesis states birds who are more generalist are more likely to exhibit morphic differences, due to disruptive selection (Galeotti & Rubolini, 2004) (chapter two). New Zealand kaka have a quite narrow niche, confined to native bush habitat, when compared to New Zealand robins which have been known to make use of a variety of habitats and lifestyles including exotic pine plantations.

#### **4.1.4 Difference in genetic markers:**

In numerous studies of bird population genetics; mtDNA sequences reveal little population differentiation compared to microsatellites. While it has been reported that many avian studies where microsatellites and mitochondrial results are compared frequently recover a pattern of reduced nuclear genetic structure (White, 2013) there are exceptions to this rule. It has been reported for instance, that it is possible to get both similar levels of genetic structure and higher levels of nuclear structure (Brito, 2007). In general microsatellites and mtDNA reveal similar patterns in population structuring through many avian taxa (Zink & Barrowclough, 2008).

MtDNA Control Region (CR) has a fast rate of evolution, and, the CR has been typically deemed to be most appropriate for intraspecific studies. But it has limited base substitution variation between individuals as haplotypes can be shared by many individuals. This implies that recent loss of genetic variation leaves little trace on mtDNA and therefore CR is a powerful tool for determining the current status of population structure and identification of subspecies and species rather than the individual identity and contemporary changes in genetic structure.

Microsatellites show high variability so that even with a small number of loci and a large number of individuals, all individuals should have a unique multi locus genotype. This makes it possible to address issues such as relationships, structure and classification, not only at the population level but also at the individual level.

Research suggests that mtDNA is a reliable indicator of geographical population structure, but it may be more advantageous to use nuclear markers as well to answer other evolutionary questions, such as resolving phylogeny and estimating coalescence times (Zink and Barrowclough, 2008).

#### **4.1.5 Aims:**

This study looked for evidence of genetic structure among New Zealand falcon populations and examined support for the proposed grouping's observed and proposed by Fox (1977) which are generally accepted as morphs presently. This study also attempted to identify if the genetic structure seen may correspond to the morphometric patterns identified in chapter two, and what geographic boundaries they may follow.

## **4.2 Methods:**

### **4.2.1 Microsatellites as useful markers:**

Microsatellites are short DNA sequence stretches in which a motif of one to six bases is repeated in tandem. It has been known for some time that these sequences can differ in repeat number among individuals. It is widely assumed that the short proto microsatellites are generated by random point mutations followed by rare slippage events that extend the short proto microsatellites (Selkoe & Toonen, 2006). Extra copies of repeats can then be formed by slipped strand mis-pairing resulting in a larger microsatellite. Over time as a population breeds by sexual reproduction microsatellites recombine, thus a population will maintain a variety of microsatellites that are characteristic for that population and distinct for others. Of the various types of molecular markers used today, microsatellites have many positive attributes, including hyper-variability, co-dominance, abundance and tolerance to variation in DNA quality and quantity (Selkoe & Toonen, 2006). Additionally, due to a reasonably good understanding of molecular evolution and the development of robust computational methods, microsatellites are well suited to answer questions related to effective population size, population structure, migration and colonization rates, and reproductive system, thus providing essential data for conservation (Banhos et al, 2008). There are approximately 100 microsatellite loci isolated and characterized for Falconiformes. Microsatellites are among the most variable types of DNA sequence in the genome. In contrast to unique DNA, microsatellite polymorphisms derive mainly from variability in length rather than in the primary sequence. Moreover, genetic variation at many microsatellite loci is characterized by high heterozygosity and the presence of multiple alleles, which is in sharp contrast to unique DNA (Ellegren, 2004).

## 4.2.2 Sample collection:

Samples of blood, feathers, muscle and toe tissue were collected from various sources and locations (table 4.1). Samples originated from throughout New Zealand except north of Auckland. Sources included The Department of Conservation, Museums, universities, captive facilities, individual researchers and bird enthusiasts. Samples from captive birds in aviaries were supplied by Wingspan Birds of Prey Trust. Tissue samples collected from muscle and toe pads from dead birds were preserved in 90% ethanol. Feathers were stored dry in plastic bags and frozen and blood samples collected under permit from live birds were stored in either 90% ethanol or Seutin's buffer. In total 122 samples were retrieved.

Table 4.1: Source of each sample type.

Source	Extracted DNA	Blood	Feather	Toe pad	Muscle
Wingspan Birds of Prey Trust		x	x	x	
DOC (offices and individual staff)			x	x	x
Canterbury museum				x	
Wildbase (Massey University)		x			
Waikato University (Tess Embling)	x				
Massey University falcon researchers		x			
Individual falcon enthusiasts			x		
Nick Fox			x		

## 4.2.3 DNA Extraction:

Museum specimens were sampled by taking a small scraping (approximately 3 mm by 3 mm) from the toe-pad using a sterile scalpel. Feathers were sampled by removing 2–3 mm of the tip of each feather. Approximately 50-100 um were taken out of the buffered samples and a 3mm by 3mm section of muscle tissue was taken from the chest of frozen whole birds. Extraction methods differed depending on the type of tissue/sample used. Extraction methods were selected and modified as appropriate for the type tissue/sample used. Experiments were conducted to determine the best method for each tissue type. A Phenol/chloroform method for feathers and either

GenElute kit (Sigma-Aldrich) or a Phenol/chloroform method was used for blood, toe pads and chest tissues.

All samples were digested through incubation at 55 C° with Proteinase K (10ng/μl) and a CTAB buffer (100ml 1M Tris, 280 ml 5 M NaCl, 40 ml of 0.5 M EDTA and 20g of CTAB) in a Total Lab Systems LTD Infors Minitron shaker at approximately 200rpm for 10-24 hours until digested. This was followed by a combined phenol, chloroform/isoamyl alcohol cleanup based on methods previously described (Sambrook et al, 1989). DNA was precipitated using 500μl of ice-cold ethanol, mixed via inversion and left in freezer overnight. After spinning at 14,000rpm for 10min, the ethanol was poured off, and after drying the DNA was re-suspended in 40μl of H<sub>2</sub>O for storage. The quantity and quality of DNA was checked using a Thermoscientific nanodrop spectrophotometer and ND-1000 V3.6.0 software. GenElute extractions were performed following manufacturer's methods. The DNA was then re-suspended in water and stored in a minus 20 freezer.

#### **4.2.4 PCR Amplification:**

##### ***MtDNA Control Region:***

A fragment of the mitochondrial Control Region (CR), approximately 525 base pairs long, was amplified. Different primer pairs were used with different quality DNA. PCR with DNA templates from modern tissue used primers L15206 (CTATGTATTACTTTGCAT) and H15856 (GTGAGGAACGGATCGAAG) previously applied to peregrine falcon (Talbot et al, 2011). Modified primer CR624 (CGACAGCCATCTATACATTCTAGGA) was designed for use with H15856 for use with lower quality templates. PCR amplifications for all samples were carried out in 10 microliter volumes using Mclab and Roche taq. Recipes contained 0.5 μl reverse and forward primers, 1 μl manufacturer's buffer, 1 μl dNTP's, 5.9 μl H<sub>2</sub>O, 0.11 μl taq, when using Roche taq 0.2 μl of Magnesium was added. All PCRs were carried out using the Biometra T3000 thermal cycler.



The PCR protocol for amplifying CR samples used the following cycling parameters- an initial denaturing temperature of 94°C for 3 min, followed by 30 cycles of: 94°C for 15 sec, 51°C for 15 sec and a 1 min extension of 72°C, this was finished with 7min final extension at 72°C. PCR products were electrophoresed on a 1% agarose gel made from Amesco agarose powder and 1x TAE Buffer (40 mM Tris acetate, 1mM EDTA pH 8.0 ) at 120V for 30min, alongside a 1Kb+ DNA Ladder (Life Technologies). PCR products were then visualised and photographed under U.V. light using a BioRad gel-doc with BioRad QualityOne 4.4.0 software. Each product was sequenced using standard protocols. Sequencing reactions were performed by the Massey University Genome service center using a capillary ABI3730 automated Genetic analyser (Applied Biosystems). Sequences were viewed and aligned in Geneious (version 6.1.7 Biomatters LTD).

#### ***Microsatellite loci:***

Twelve primer pairs that have been successful in genotyping peregrine falcon (Nesje et al, 2000) were trialled using the M13-tailed primer method where forward primers were 5'-tailed with the 23 base pair M-13 sequence (Boutin-Ganache et al, 2001). The New Zealand falcon samples chosen for microsatellites analysis were chosen for their geographical diversity. Loci were PCR optimized and tested by amplification with DNA from these individuals. A positive control (Australian peregrine) was used. The forward primer was labelled with 6-FAM or HEX dyes (Invitrogen Applied systems). This method of adding the fluorescent dye to the PCR reaction was not ideal; while the PCR worked resulting in clean bands seen on 1% agarose gels the genotyping was unsuccessful.

A new method was used, where the primers were purchased with fluorescent dye already attached (Invitrogen Applied systems). The forward primer of each pair identified as the most successful in the first method was labelled with HEX and 6-FAM (NVH fp5, NVH fp13, NVH fp31 and NVH fp79-4). PCR reactions were performed in 10 µl reaction volume containing 1µm of genomic DNA. Each reaction mixture included 0.1 Taq (Mclab), 1 µl manufacturer's PCR buffer, 0.5 µl of each labelled primer, 1 µl of each dNTP and 5.9µl H<sub>2</sub>O. The protocol set for amplification was as follows: an initial denaturing of 94°C for 3 min followed by a cycle of 94°C for 30 sec, a 15 sec annealing

period set at 50°C and a 45 sec extension at 72°C, finished by a 5 min final extension set at 72°C. Products were pooled before sending for genotyping as a cost saving measure. Products with >50bp and the same fluorescent dye were combined, as were those with 20 base pair difference. The microsatellite loci were amplified by PCR using a Biometra T3000 thermal cycler and ABI genotyping used GeneScan-500 LIZ size standard for fragment sizing. Once the method was optimized, a further two labelled primers were purchased, NVH fp82-1 and NVH fp46-1 to complete the dataset (table 4.2).

Table 4.2: Microsatellite primer pairs for each loci used in the analysis originally isolated in peregrine falcons (*F.peregrinus*) (Nesje et al, 2000).

<b>NVH fp5</b>	F: CCGTTCTGGAGTCAAAC	R: CATGCAGCACTTATTTCAG
<b>NVH fp13</b>	F: AGCTTGATTGAGGCTGTG	R: CCAAATTCCTGCTGAAG
<b>NVH fp31</b>	F: ATCACCTGCACATAGCTG	R: TTTAGCTCCTCTCTCAC
<b>NVH fp46-1</b>	F: TTAGCCTCGCAGCTTCAG	R: GTAATGAAAAGTCTTTGGGG
<b>NVH fp79-4</b>	F: TGGCTTCTTTATCAGTAAC	R: GGCTGGGTGGAATTAAG
<b>NVH fp86-2</b>	F: GTAAATAAGCCTCCAAAAGG	R:CATGCTTCCTGATTCTC

#### 4.2.5 Analysis:

##### ***Mitochondrial Control Region:***

Geneious 6.1.7 (Biomatters Ltd) was used to align sequences and used to trim the sequence ends. The final alignment was imported from Geneious (Biomatters Ltd) and the trait network created by putting each bird's location into one of 10 possible regions within New Zealand. Originally 74 sequences were aligned however 22 of these were only 425 base pairs long. For the final analysis these were taking out as they skewed the network when retained. The final analysis therefore contained 52 sequences. PopArt (Allan Wilson Centre Imaging Evolution Initiative; <http://popart.otago.ac.nz>) was used to create a haplotype network from the data using an integer neighbour joining method

***Microsatellite loci:***

Microsatellites were imported into Genemarker (v 6.0) (Soft genetics) software for scoring. To determine if the microsatellite data would show any structure throughout the country and whether the structure may correspond to the morphological structure found. STRUCTURE (version 2.3.4) was used to analyse the microsatellite data. In total 6 loci and 47 individuals were run through STRUCTURE. A burnin period of 10,000 repetitions was requested and 100,000 repetitions MCMC after burnin. The number of clusters (K) was set at 1-15 in separate runs with 10 iterations for each. We wanted to test for the current morph proposal (K=3) as well as any other possible number of populations. Values of K=1 indicate a genetically uniform population, while values of K=2 and so on indicate the existence of genetically different arrays of individuals. The outputs were analysed in the online programme Structure Harvester (v 0.6.93) (<http://taylor0.biology.ucla.edu/>). The averaged K within the dataset was compared using the Evanno method (Delta-K), a graph and table were produced to understand the most likely number of clusters found within the dataset and the most likely natural groupings, if any. The popfiles and indfiles were taken from Structure Harvester (v 0.6.93) and put into CLUMPP (v 1.1.2) (Rosenberg lab, Stanford University) for further analysis, Paramfiles were adjusted for 47 individuals, 15 populations and 10 iterations. This merges the individual data for each individual and each population over the 10 iterations. The output imported into DISTRICT (v 1.1) (Rosenberg lab, Stanford University) to create the bar plots of each putative K value.

To investigate for any signal of population structuring corresponding to the original proposal of three morphs and the two morphs hypothesis (chapter 2) ARLEQUIN (v 3.5.1.2) was used to perform an AMOVA analysis. The data was grouped in alternative ways reflecting the different hypotheses of the population structuring and the AMOVA repeated with each (figure 4.2). Firstly, six groups were created based on current morph proposal, the three main groups were based on the location for each individual (Bush, Eastern and Southern locations) and each of these groups was broken into two.

Group 1- Bush (North Island individuals) and West Coast (west coast of the South Island individuals)

Group 2 – Eastern (Individuals from eastern South Island) and Southland (individuals from Otago and Southland)

Group 3 – Southern (Fiordland and Auckland Islands).

We used the hierarchical analysis of molecular variance to test for the overall significance of genetic differentiation among these three regional groups (FCT), among populations within these regions (FSC), and among populations (FST).

Data were then grouped into four regions, based on the morphological results from chapter two, the North Island was split into upper North Island and lower North Island and the South island was split into upper South Island and lower South Island. Finally, the data was partitioned into 15 populations corresponding to 15 regions as seen in the Structure analysis, to look into possible structure at a finer level.

The extent of genetic differentiation between populations was quantified using a distance by isolation analysis. Pairwise FSTs were calculated in ARLEQUIN (AMOVA analysis) and Geographic distances between individual sampling locations were retrieved from Google Earth (v 6.0) (Google Inc.). Geographic distance was interpreted as the straight-line distance between the centres of the different sampling areas in kilometers. A Mantel test was carried out to test between the genetic differentiation and geographical distances in ARLEQUIN run with 1000 permutations. The pairwise FSTs ( $F_{ST} / (1 - F_{ST})$ ) of all individual falcons (Auckland islands not included) were also graphed against the natural log of the geographic distances for visual analysis of the correlation.

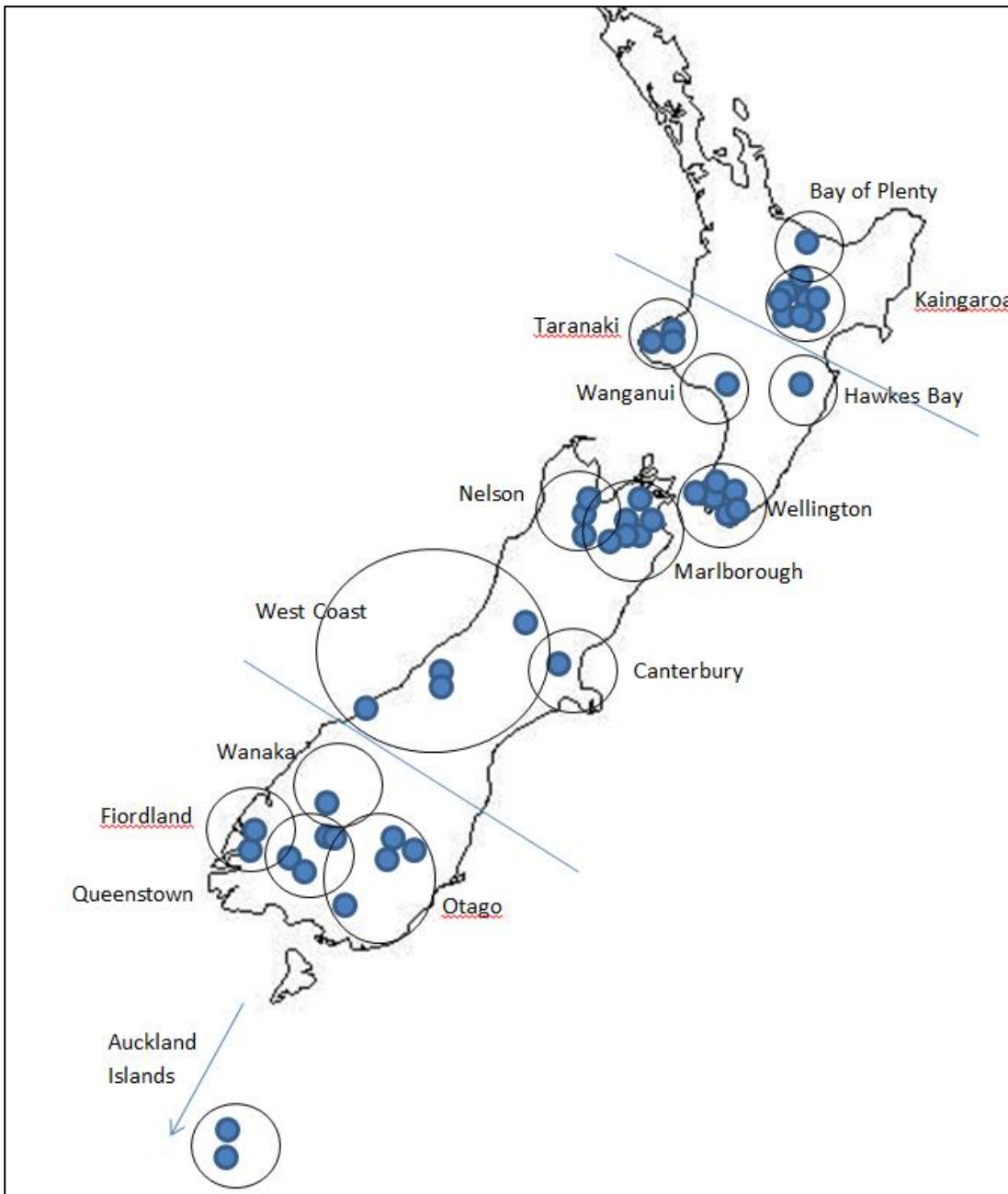


Figure 4.2: Locations of samples for microsatellite analysis. Regions circled correspond to the 15 regions used in the analysis. Blue line represents divisions for upper North Island and lower North Island and upper South Island and lower South Island.

## **4.3 Results:**

In total 122 samples were retrieved, however not all were used in the analysis; many contained low quality DNA and were unable to be used. There are differing sample sizes for CR analysis and microsatellite analysis, in many cases the initial sample size has reduced after the DNA quality was examined and was deemed too poor for amplification. A total of 74 individuals were sampled from 15 New Zealand regions for CR analysis and 47 were used for microsatellite analysis (figure 4.2).

### **4.3.1 MtDNA Control Region:**

Control Region sequences were obtained from 74 New Zealand falcons which included 52 sequences of 500 base pairs long and 22 that were 400 base pairs long. Shorter sequences resulted from primers CR624 and H15856 applied to DNA of lower quality. Haplotype networks with different combinations of these different sequences were trialled, breaking locations into 10 or 15 regions throughout New Zealand. When the shorter sequences were added into the network; no dramatic differences were seen. They only boosted the numbers of individuals within the haplotypes already recognized. Genetic diversity was low, with just five haplotypes identified throughout New Zealand, differing by no more than two point mutations. The most common haplotype was found throughout all regions of New Zealand (figure 4.3). Two haplotypes were only found in the North Island in Kaingaroa forest and the Central North Island; both are only one base substitution different from the most common haplotype. Another haplotype, two substitutions from the most common haplotype, was found only on the top of the South Island specifically Marlborough and Nelson.

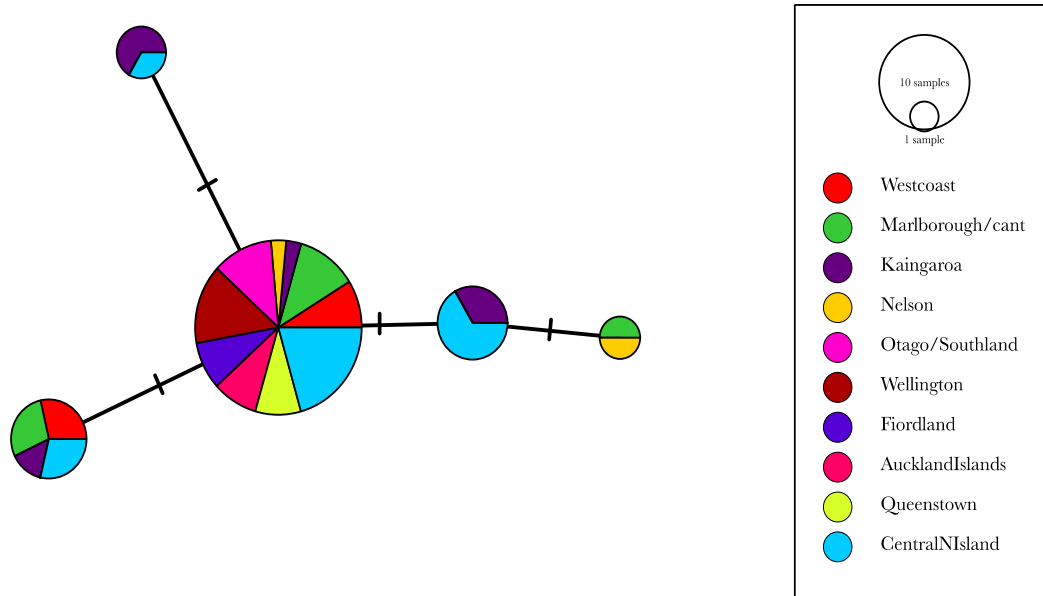


Figure 4.3: Haplotype network showing genetic distances between mtDNA control region haplotypes in New Zealand falcon (n=52) over 10 regions around New Zealand. Five haplotypes were identified, with one common haplotype found over all 10 regions. Colours represent the sample location/individual, and the size of the circles is scaled by the sample size.

### 4.3.2 Microsatellites:

Genotypes for 47 falcons from all 6 loci were obtained. The number of alleles per locus ranged from between 2 and 13 alleles and the observed heterozygosity from 0 to 44% (table 4.3). There was low heterozygosity across the entire country with the observed heterozygosity being lower than expected at each locus. Expected heterozygosity ranged from 0.14 to 0.85 while the observed heterozygosity ranged from 0.0 to 0.45.

Neither the Structure nor AMOVA (ARLEQUIN v 3.5.1.2) analyses provided evidence of genetic structure in the microsatellite data within the New Zealand falcon population. Structure harvester showed peaks at K=2 and at K= 5 using the EVANNO method seen in the Delta (K) graph (figure 4.4). Bar plots created in Structure show no apparent support for K=2 and K=3 and K=5 (figure 4.5). It is highly likely from this that the true K is K=1. Structure does not assess K=1, but when we see mixed membership

proportions among groups, and when individuals are not clearly assigned to any one group, it is very likely that no population structure is present in the data (Kalinowski, 2011).

Table 4.3: The number of alleles, observed and expected heterozygosity for each microsatellite locus examined in the New Zealand falcon population.

Locus#	Gene copies	Number alleles	Observed Heterzygosity	Expected Heterozygosity
Fp 5	94	3	0.00000	0.23244
fp 31	94	4	0.44681	0.66003
fp 13	94	13	0.21277	0.85267
fp 79-4	94	5	0.29787	0.61016
fp 86-2	94	2	0.00000	0.25623
fp 46-1	94	4	0.06383	0.14299

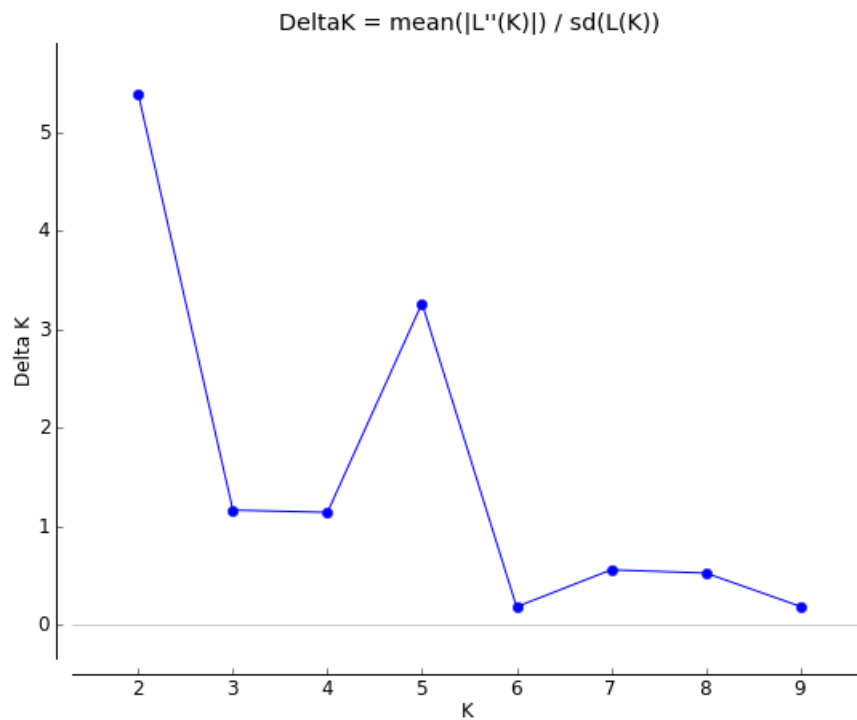


Figure 4.4: Evanno et al (2005) plot for detecting the number of K groups that best fit the data.



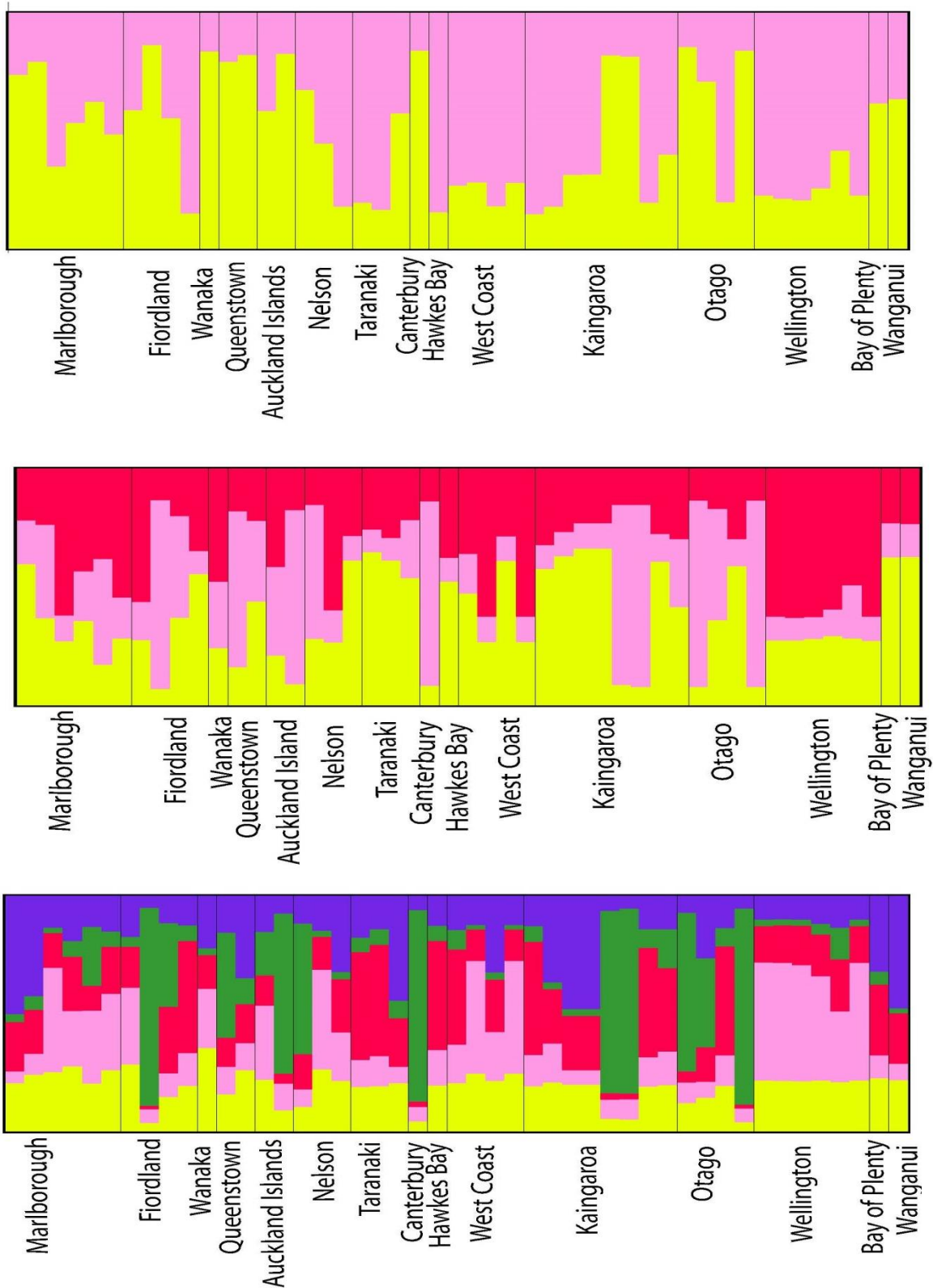


Figure 4.5: Population structure of New Zealand falcons (n=47) based on six microsatellite loci from 15 sampling regions throughout New Zealand. Graphs show average results of 10 Structure iterations for K=2 (top), K=3 (center) and K=5 (bottom).

A hierarchical analysis of molecular variance of population structure revealed no significant subdivision between the populations/regions of the currently accepted morphs Bush, Eastern and Southern or among populations within the regions. Most variation (87%) was found within the populations ( $F_{ST}=0.12990$  ( $p=.002$ )) (table 4.4). The analysis was repeated using the North and South groupings of sample data (table 4.5), and this too returned no significance between regions. There was however, a significant difference between populations within the regions ( $F_{SC}=0.08135$  ( $p=0.005$ )) and again within the populations ( $F_{ST}=0.09249$  ( $p\text{-value}=0.001$ )). A similar pattern was seen when the data was broken into four groups (upper north, upper south, lower north and lower south). Each time the majority of variance was attributed to within the populations not between them.

Table 4.4: A hierarchical analysis of molecular variance (AMOVA) of falcon microsatellites between Bush, Eastern and Southern morphs.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	2	9.468	0.02120 Va	1.49
Among populations within groups	3	9.318	0.16422 Vb	11.50
Within populations	88	109.300	1.24205 Vc	87.01
Total	93	128.085	1.42747	

Fixation Indices

FST : 0.12990

FSC : 0.11678

FCT : 0.01485

Significance tests (1023 permutations)

Vc and FST :  $P(\text{rand. value} < \text{obs. value}) = 0.00293$

$P(\text{rand. value} = \text{obs. value}) = 0.00000$

P-value = 0.00293+-0.00164

Vb and FSC :  $P(\text{rand. value} > \text{obs. value}) = 0.10655$

$P(\text{rand. value} = \text{obs. value}) = 0.00098$

P-value = 0.10753+-0.01069

Va and FCT :  $P(\text{rand. value} > \text{obs. value}) = 0.26100$

$P(\text{rand. value} = \text{obs. value}) = 0.05083$

P-value = 0.31183+-0.01393

Table 4.5: A hierarchical analysis of molecular variance (AMOVA) of falcon microsatellites between the North Island (Upper and Lower) and South Island (Upper and Lower).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	4.698	0.01715 Va	1.21
Among populations within groups	2	7.882	0.11365 Vb	8.04
Within populations	90	115.506	1.28339 Vc	90.75
Total	93	128.085	1.41420	

Fixation Indices

FST : 0.09249

FSC : 0.08135

FCT : 0.01213

Significance tests (1023 permutations)

Vc and FST : P(rand. value < obs. value) = 0.00196

P(rand. value = obs. value) = 0.00000

P-value = 0.00196+0.00136

Vb and FSC : P(rand. value > obs. value) = 0.00587

P(rand. value = obs. value) = 0.00000

P-value = 0.00587+0.00219

Va and FCT : P(rand. value > obs. value) = 0.33333

P(rand. value = obs. value) = 0.34800

P-value = 0.68133+0.01340

Some  $F_{ST}$  values indicate a significant departure from zero that is consistent with genetic partitioning between site samples and grouped samples. Population pairwise  $F_{ST}$ s (AMOVA) structured according to the traditional three morph hypothesis suggested significant differences between groups 1 (North Island and West Coast of South Island) and 2 (Eastern South Island and Southland) and groups 1 and 3 (Fiordland and Auckland Islands). This suggests genetic partitioning between the Bush and the Eastern and Southern morphs. Pairwise  $F_{ST}$ s also suggest some significant differences when the data was divided into six populations (table 4.6). This result suggested the North Island population is significantly different from the Southland, Fiordland and Auckland Island groups, and the West Coast is different from the Southland and Auckland Island groups. In general the North Island population appears to be significantly different from the far south of the South Island. When simply broken into the upper north/upper south and lower north/lower south (table 4.7), the lower south and upper north island are significantly different. Along with this the upper south and lower south show significant difference in pairwise  $F_{ST}$ s as well.

When considered as 15 sampling groups (table 4.8) we see the difference in more detail- The Wellington region is significantly different to most Southern populations, as well as Marlborough and Kaingaroa. There is a private allele only present in the Wellington region and Kaingaroa forest. The Southern areas (Wanaka and Fiordland) also have some private alleles. However it is difficult to see any clear pattern given the low sample size and the low number of loci used in the analyses. Some populations were represented by only one individual, making it difficult to gain any statistical significance.

Table 4.6: Population pairwise FSTs calculated from 6 microsatellite loci. Numbers indicate sample locality (1= North Island, 2= West Coast (SI), 3=Eastern (SI), 4=Southland, 5=Fiordland and 6=Auckland Islands) of New Zealand falcon morphs Bush, Eastern and Southern (n=47). Sample size, morph (North or South Island and Bush, Eastern or Southern) and significant FSTs (bold) are given.

	<b>N</b>	<b>Morph</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
1	20	NI/B	0.00000					
2	4	SI/B	0.07280	0.00000				
3	10	SI/E	0.04683	0.08936	0.00000			
4	9	SI/E	<b>0.17408</b>	<b>0.27777</b>	0.10991	0.00000		
5	2	SI/S	<b>0.13965</b>	0.17526	0.10662	0.05633	0.00000	
6	2	/S	<b>0.30782</b>	<b>0.40000</b>	0.19741	0.13013	0.14185	0.00000

Table 4.7: Population pairwise FSTs calculated from 6 microsatellite loci. Numbers indicate sample locality (1=Lower North Island, 2=Upper North Island, 3=Upper South Island, 4= Lower South Island) of New Zealand falcon (N=47). Sample size, morph (North or South Island and Bush, Eastern or Southern) and significant FSTs (bold) are given.

	<b>N</b>	<b>Morph</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
1	11	NI	0.00000			
2	9	NI	0.04090	0.00000		
3	14	SI	0.04904	0.04277	0.00000	
4	13	SI	0.15264	<b>0.08474</b>	<b>0.10369</b>	0.00000

Table 4.8: Pairwise FSTs calculated from 6 microsatellite loci among 15 populations of New Zealand falcon (n=47). Numbers indicate sample locality ordered approximately north to south (1-Taranaki, 2-Wanganui, 3-Bay Of Plenty, 4-Wellington, 5-Hawkes Bay, 6-Kaingaroa, 7-Westcoast, 8-Marlborough, 9-Canterbury, 10-Nelson, 11-Otago, 12-Queenstown, 13-Fiordland, 14-Wanaka and 15-Auckland Islands). Sample size, morph (North or South Island and Bush, Eastern or Southern) and significant FSTs (bold) are given.

	N	Morph	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	3	NI/B	0.00000														
2	1	NI/B	0.31429	0.00000													
3	1	NI/B	0.38318	0.40000	0.00000												
4	6	NI/B	0.29480	0.49623	0.61387	0.00000											
5	1	NI/B	0.21053	0.71429	0.80000	0.34513	0.00000										
6	8	NI/B	-0.00609	0.28772	0.40632	<b>0.16789</b>	0.24306	0.00000									
7	4	SI/B	0.23937	0.47774	0.63470	<b>0.14528</b>	0.34247	0.12469	0.00000								
8	6	SI/E	0.07309	0.14317	0.29632	<b>0.15871</b>	0.16482	0.06601	0.07828	0.00000							
9	1	SI/E	0.40000	0.75000	0.83333	0.58056	1.00000	0.32368	0.64964	0.18720	0.00000						
10	3	SI/E	-0.01667	0.31692	0.40000	0.16184	0.18777	-0.05243	0.00500	-0.03903	0.34276	0.00000					
11	4	SI/E	0.13296	0.38723	0.31814	<b>0.38388</b>	0.23632	0.16369	0.35782	0.16204	0.14024	0.12227	0.00000				
12	4	SI/E	0.22336	0.07834	0.17489	<b>0.35294</b>	0.26638	0.21040	<b>0.32686</b>	0.09091	0.21127	0.14749	0.07807	0.00000			
13	2	SI/S	0.10707	0.30051	0.30567	<b>0.26865</b>	0.13271	0.10833	<b>0.24424</b>	0.13197	0.25202	0.10278	0.03429	0.11302	0.00000		
14	1	SI/E	0.67391	0.80000	0.88889	0.73922	1.00000	0.64304	0.74054	0.47988	1.00000	0.64023	0.56237	0.52941	0.52798	0.00000	
15	2	N/S	0.35135	0.51515	0.52941	<b>0.48583</b>	0.56364	0.30001	0.40000	0.20727	0.44186	0.19836	0.17590	0.16981	0.21172	0.68000	0.00000

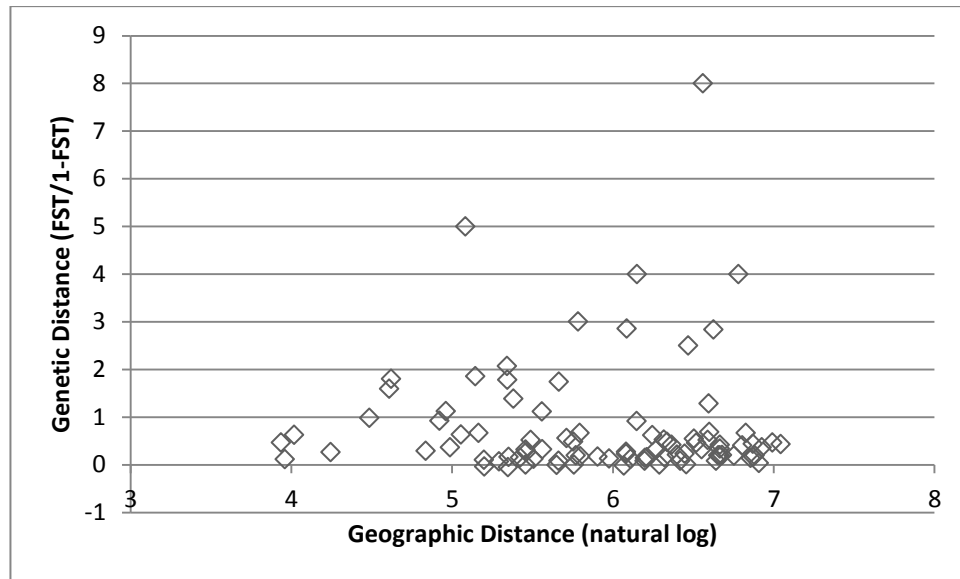


Figure 4.6: Genetic distance (pairwise FSTs) plotted against geographic distance (km).

Pairwise FSTs show some significant differences between some regions of New Zealand with significant difference in the North Island and those in the far south of the South Island (table 4.8). Visual interpretation may suggest a pattern of isolation by distance (figure 4.6) but a Mantel test (ARLEQUIN v 3.5.1.2) revealed no significant correlation between geographic distance and genetic distance ( $p=0.713000$ ).

Combining the mtDNA and microsatellite data analysis together we can see some structure between the North and South Islands can be seen with some unique haplotypes only found in one or other of the main islands. Two haplotypes are present only in the North Island, and one only in the South Island. Structure software did not identify distinct population clusters in microsatellite loci but when placing individuals in regional groups ARLEQUIN (v 3.5.1.2) identified some differences between the regional populations in New Zealand.



## **4.4 Discussion:**

### **4.4.1 Genetic support for morphs:**

Mitochondrial and microsatellite data are consistent in revealing minimal genetic structuring in the New Zealand falcon, with no support for the original three morph proposal. The mtDNA control region showed a small amount of structuring with apparently private haplotypes present in the North and South Islands; however increased sampling might show otherwise. Analysis of microsatellites using STRUCTURE did not support regional clusters. However, when individuals were grouped by regions using ARLEQUIN significant differences among some of the regions based on pairwise  $F_{ST}$ s were present, suggesting some structuring among the regions. It is likely that private alleles/haplotypes may be unique to a locality rather than to an 'island'. Both Wellington and regions to the far south (Fiordland) possessed private alleles; and it is most likely this trend that the AMOVA analysis was identifying. Unfortunately, we cannot draw firm conclusions from these results with such a small sample size and because some of the regions were only represented by one sample. Most variation in the analysis was present within the populations and not between groups. Consequently, there is no correlation of genetic structure with either the three morph or North Island/ South Island hypotheses. Reasons for this and comparisons with other species are explored in the following sections.

### **4.4.2 Comparison to other falcon species:**

It is possible to identify the population differentiation in some island species of falcon at microsatellite level such as the common kestrel on the Cape Verde Islands and the Scandinavian peregrines. Peregrine falcons, which have been studied in detail, exhibit low variation in mtDNA Control Region; however, significant differences in some populations can be seen using microsatellite analysis. Research on peregrine falcons in Scandinavia and surrounding areas found significant difference in microsatellite data

between northern and southern populations, as well as a significant difference between Scandinavia and nearby Scottish populations (Nesje et al, 2000). However, other continental falcons seem to show much less differentiation. In saker falcons, subspecies designations are all based on morphology, with little correlation to genetic data. For example, the Altai falcon from the mountainous area of central Asia was once thought to be a different species based on morphology, but really is a morph of saker falcon based on genetic analysis (Cade, 1982). This interpretation is confirmed by hierarchical AMOVA, in which 96% of the genetic variance could be attributed to variation within populations (Nittenger et al, 2007), a very similar result to the variance this analysis found within New Zealand falcons.

#### **4.4.3 Comparison to other New Zealand birds:**

The kereru/wood pigeon (*Hemiphaga novaeseelandiae*) has a similar pattern of population structuring to the New Zealand falcon with one very common haplotype and many singletons in the mtDNA Control Region. The kereru, however, has higher genetic variation (Goldberg et al, 2011). Like the falcon, kereru show little spatial structure throughout the country. Goldberg et al (2011) had a slightly larger sample size, with 67 individuals compared to the 52 analysed here. Their genetic analysis identified 23 different haplotypes across mainland New Zealand, of which 19 were singletons, along with four from the Chatham Islands and two from Norfolk Island. The lack of spatial structure in kereru populations may be explained by a high level of movement throughout the species range. New Zealand falcons are also likely to display a high level of movement, but, they are known to show philopatric behaviour in adult hood (Fox, 1977). A lack of information on the natal dispersal of New Zealand falcons hinders the understanding of ecological dispersal, although it is speculated that this is the time when movement among populations is likely.

The Blue Duck (*Hymenolaimus malacorhynchos*) shows a far stronger spatial structure than the Kereru and New Zealand falcon. Among 78 blue duck samples, 12 mtDNA control region haplotypes were identified, which formed two distinct clades that

correspond to populations in the North Island and the South Island (Robertson et al, 2007). The genetic differentiation in this case, is attributed to allopatric fragmentation and restricted gene flow; however, this is based on mtDNA alone. Little is known about blue duck dispersal but they are likely to be less mobile than falcons. There has been some evidence of natal philopatry observed but (Robertson et al, 2007), like the New Zealand falcon, very little is known about the juvenile dispersal of the species.

#### **4.4.4 Importance of dispersal in population structuring:**

Dispersal is a significant life-history trait of vagile species that affects the distribution and genetic structure of populations. Natal dispersal in birds is the movement of an individual from its hatch site to a new location where its first reproductive effort occurs (Penteriani & Delgado 2009, Greenwood 1980). Breeding dispersal is movement of an individual from a nesting site to a new site over different breeding seasons (Greenwood, 1980). This distinction may be quite important in the New Zealand falcon, where the traditional view is that they are highly philopatric. Research on raptor dispersal in general usually shows a female biased dispersal, which is related to differences in gender roles in territory acquisition and defence (Dennhardt & Wakamiya, 2013). Typically, more females than males disperse or they disperse farther than males. This could be related to the amount of effort each sex expends competing for territories or mates versus the amount of effort spent in raising young (Dennhardt & Wakamiya, 2013). The assumption is that males gain foraging and anti-predator benefits that improve reproduction and survival when establishing territories in familiar areas. So, if males are philopatric or attached to their territories then females need to disperse further to avoid inbreeding and to search for mates (Restani & Mattox, 2000). There is also evidence that individuals tend to become more philopatric with increasing age, lessening breeding dispersal (Dennhardt & Wakamiya, 2013). This makes sense in terms of the increased benefits from breeding site familiarity which is expected to be beneficial to survival.

From this, it can be predicted that those species with high philopatry may lower their genetic variation through less movement between groups resulting in less gene flow. Limited natal dispersal is going to encourage population variation/differentiation through limiting gene flow between falcon populations (Alcaide et al, 2009). Long-term and extensive ringing studies of Lesser Kestrels in Spain have documented high natal and breeding philopatry as well as a negative association between effective dispersal and geographical distance (Alcaide et al, 2009). When comparing the genetics of lesser kestrels with the less philopatric and wider distributed Eurasian kestrel, it is hypothesised that the Lesser Kestrel would have lower genetic variation. However, it was found that both species had similar levels of genetic variation in seven microsatellite loci. So this questions whether philopatric behaviour has any impact on genetic diversity or population structure.

Dispersal in New Zealand falcons is poorly understood. Many believe that falcons are sedentary and philopatric, however it is known that the initial movements of juveniles may be far reaching. Research by Seaton (2008) showed mean natal dispersal of radio tracked individuals in Kaingaroa forest was 9.6 kilometers, with no significant difference between males and females. Individuals were radio tracked out of the nest and only four females were successfully tracked for 91 days after fledging. The remaining birds travelled too far for successful radio tracking suggesting that individuals may be moving large distances away from the hatch site (Seaton et al, 2008). There are reported cases of juveniles remaining near hatch sites throughout consecutive years (pers. obs.) but these seem to be exceptions to the general rule. The only satellite tracking of New Zealand falcons was an adult female monitored for three years in Kaingaroa forest. She remained near her nesting territory the entire time and the furthest distance travelled was 137 kilometers (Holland & McCutcheon, 2007). With only limited dispersal data it is difficult to draw robust conclusions as to the dispersal of New Zealand falcons although the evidence suggests that individuals may be sedentary or highly mobile. Recent research on juvenile saker falcons has shown that they are capable of travelling very long distances from their hatch sites (Gamauf & Dosedel, 2012). Evidence from the neutral genetic markers in this analysis suggests

that New Zealand falcons do cross between the North and South Islands, raising the question of why there are two distinct size morphs present.

#### **4.4.5 Habitat effects:**

There is some evidence that the New Zealand falcon may show a habitat dispersal bias resulting from philopatry, for example juveniles dispersing to habitat they have been raised in e.g. 'Bush' falcons to bush habitat and 'Eastern' falcons to open habitat (Fox, 1977). But there is little known as to the genetic consequences for the population. Habitat biased dispersal has been found to strongly influence genetic structure among populations of island birds such as the Galapagos warbler finches (*Certhidea olivacea* and *Certhidea fusca*) (Tonniss et al, 2005). But, there has been little interest in examining the landscape scale population genetic structure of highly vagile organisms such as falcons because of the assumption that the high levels of gene flow would prevent population genetic structure at smaller spatial scales. This assumption would be valid if gene flow were spatially random (Manel et al, 2003). However, a host of recent studies on wide-ranging terrestrial vertebrates indicate that this often is not the case and that populations can exhibit high levels of structure at the landscape scale in particular. Habitat has been identified as an important determinant of population genetic structure in wide-ranging habitat generalists even where no physical barriers exist. Hull et al (2008) for example, found that the red tailed hawk shows evidence of morphological and genetic structure based on habitat preferences (refer to chapter two). Hull et al (2008) identified a pattern of isolation by distance, but that also corresponded with habitat breaks between eastern and western slopes of the Sierra Nevada. This analysis found no significant evidence of isolation by distance; however it may be difficult to pick up such patterns with the small sample size in the analysis. If there truly is no isolation by distance within the New Zealand falcon population it suggests that there is movement within the population creating gene flow. This makes sense given the suggestion of high juvenile movement in New Zealand falcon.

#### **4.4.6 Conclusion:**

Two size morphs of the New Zealand falcon have been identified partitioned by the North and South Islands; however this analysis found little support genetically for this separation based on the results of neutral genetic markers. Evidence from this analysis suggests that the falcons are responding to particular environmental conditions throughout the two islands resulting in a change in size, however high juvenile dispersal may be preventing the partitioning of gene flow between the North and South Islands. However, it is possible that with more sampling and a greater number of markers, a higher level of population structure may be identified.

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## Chapter 5:

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### **Conclusion and Conservation Implications:**



Male New Zealand falcon chick, Marlborough (Photo-Lena Olley).

Evidence from this research does not support the need for managing three distinct populations of New Zealand falcon. I did however identify distinct morphological populations correlating with the North and South Islands. New Zealand falcons located in the North Island had significantly shorter wings than those falcons in the South Island. While no significant population genetic structuring was identified in neutral markers there was some structuring present in the population. Larger sample sizes and an increase in marker numbers may shed more light on this in the future. The low level of variation and lack of partitioning suggests a recent origin of morphological differences in populations.

The largest barrier for juvenile dispersal in falcons in New Zealand is likely to be geographical barriers such as the Cook Strait. Beyond this, habitat differences, philopatric behaviour and other behaviours such as nest imprinting may play a role in the morphological differences arising in the populations throughout the North and South Islands. It may be that falcon size is a response to a gradient of environmental conditions corresponding with Bergmann's rule, but this may be partially countered by movement within the islands at the juvenile stage to average out the size variation within the islands, resulting in two distinct size morphs.

Estimating genetic divergence among populations is crucial for the conservation of many threatened and endangered species (Allendorf & Luikart, 2007). The Zealand falcons population structure has always been poorly understood and being classed as taxonomically indeterminate/data deficient (Robertson et al, 2013) has meant that they are without a conservation management plan. There are few guidelines on managing New Zealand falcons, as there is currently no Department of Conservation Recovery Group (pers. comm.). We know a number of New Zealand birds show North Island and South Island polymorphism; and that these are usually managed as distinct populations by the Department of Conservation.

Many management programs are concerned with resolving taxonomic uncertainties among groups in order to prioritize the conservation efforts below the species level

(e.g. subspecies and evolutionary significant units), which is important because much of the existing taxonomy may not reflect the underlying genetic diversity present (Hass et al, 2009). Unfortunately, basic information on population genetic structure is lacking for many threatened species, hindering conservation and management actions such as translocations and reintroductions (Moritz, 1999).

New Zealand conservation managers have used translocations and reintroductions to assist the recovery of many endangered species (Griffith et al 1989, McLean & Armstrong 1995). Currently New Zealand falcons are bred and released into the wild at only a few sites around New Zealand (Wingspan Birds of Prey Trust, Marlborough Falcon Conservation Trust). Wild falcons are rarely moved except if injured and there is a need for medical attention/rehabilitation and then returned to the wild.

Translocation of individuals among extant populations is an important tool in species conservation that allows managers to supplement dwindling populations and potentially alleviate the deleterious effects of inbreeding. Ideally, translocations should reflect the historical relationships among populations in order to avoid disruption of existing population subdivision and local adaptation (Avice 1989, Storfer 1999).

Managers of blue duck (*Hymenolaimus malacorhynchos*) have advised to only translocate individuals around neighbouring populations and not between the North and South Islands due to the presence of two genetically distinct populations (Robertson et al. 2007). Blue ducks are clearly less mobile than New Zealand falcons, generally staying within their catchment area, but like New Zealand falcons little is known on the juvenile dispersal of the species (Robertson et al, 2007). To conserve any local adaptation within the Blue duck populations, managers have been advised to only translocate individuals from within these catchment areas and neighbouring regions (Robertson et al, 2007). New Zealand falcons may also show local adaptation within the North and South Islands (even with the flexible ecology they seem to possess), supporting the current management of not translocating between the North and South Island populations.

There is also a need to revise population size estimates in the New Zealand falcon, currently the estimates are for each of the three originally proposed morphs (Bush, Eastern and Southern) and subsequently the Southern morph is reported as having very low numbers. Population numbers should be reassessed for North and South Island populations. Further work in falcon population structure may include genetic analysis with larger sample sizes and a larger number of loci and research into juvenile movement using satellite transmitters to determine the extent and timing of natal dispersal. New Zealand falcons have low genetic diversity but expanding the genetic research will provide a better picture of the possible structure within the species and morphological and genetic work with recent fossil falcon bones could be used to gain an insight into the evolutionary history of the New Zealand falcon informing on past genetic diversity, changes in shape and gene flow.

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