Detecting inbreeding depression in a severely bottlenecked, recovering species: the little spotted kiwi (*Apteryx owenii*)

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Abstract

Population bottlenecks reduce genetic variation and population size. Small populations are at greater risk of inbreeding, which further erodes genetic diversity and can lead to inbreeding depression. Inbreeding depression is known to increase extinction risk. Thus, detecting inbreeding depression is important for population viability assessment and conservation management. However, identifying inbreeding depression in wild populations is challenging due to the difficulty of obtaining long-term measures of fitness and error-free measures of individual inbreeding coefficients.

I investigated inbreeding depression and our power to detect it in species that have very low genetic variation, using little spotted kiwi (*Apteryx owenii*) (LSK) as a case study. This endemic New Zealand ratite experienced a bottleneck of, at most, five individuals ~100 years ago and has since been subjected to secondary bottlenecks as a result of introductions to new predator-free locations. There is no behavioural pedigree data available for any LSK population and the status of the species is monitored almost exclusively via population growth. I conducted two seasons of field work to determine hatching success in the two LSK populations with the highest and lowest numbers of founders; Zealandia Sanctuary (40 founders) and Long Island (two founders). I also used simulation-based modelling to assess the feasibility of reconstructing pedigrees based on individual genotypes from LSK populations to calculate pedigree inbreeding coefficients. Finally, I used microsatellite genotypes to measure the genetic erosion in successive filial groupings of Long Island and Zealandia LSK as a result of their respective bottlenecks, and tested for inbreeding depression on Long Island.

Hatching success was significantly lower on Long Island than in Zealandia in both years of the study despite significantly higher reproductive effort on Long Island. Although this was suggestive of inbreeding depression on Long Island, simulation results showed that constructing a pedigree for any LSK population based on the genetic markers and samples currently available would lead to inaccurate pedigrees

and invalid estimates of individual inbreeding coefficients. Thus, an alternative method of detecting inbreeding and inbreeding depression was required. Microsatellite data showed continued loss of heterozygosity in both populations, but loss of allelic diversity on Long Island only. Individual genotypes indicated that the majority (74%) of the adult Long Island population is comprised of the founding pair (F) and their direct offspring (F1) rather than birds from subsequent generations (F2+). This is not what would be expected if survival was equal between these two filial classes. I suggest that the high levels of inbreeding (≥ 0.25) in F2+ birds is impacting on their survival, creating a demographic skew in the population and resulting in lower hatching success on average on Long Island when compared to the relatively outbred *Zealandia* birds. This inbreeding depression appears to have been masked, thus far, by positive population growth on Long Island resulting from the long life span of LSK (27-83 years) and continued reproductive success of the founding pair. Thus, it is likely that the Long Island population will go into decline when the founding pair cease to reproduce.

This study highlights the challenges of measuring inbreeding depression in species with very low genetic variation and the importance of assessing the statistical power and reliability of the genetic tools available for those species. It also demonstrates that basic genetic techniques can offer valuable insight when more advanced tools prove error-prone. Monitoring vital rates such as hatching success in conjunction with genetic data is important for assessing the success of conservation translocations and detecting potentially cryptic genetic threats such as inbreeding depression. My results suggest that LSK are being affected by inbreeding depression and that careful genetic management will be required to ensure the long-term viability of this species.

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“There are no hopeless cases, only people without hope and expensive cases.”

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Whatungarongaro te tangata toitū te whenua

As man disappears from sight, the land remains - Māori proverb

Table of contents

Abstract	i
Acknowledgements	iii
Chapter 1: Introduction	1
Bottleneck to extinction vortex – the role of genetics in extinction.....	2
Measuring inbreeding.....	6
Microsatellite markers for conservation genetics.....	8
Conservation translocations.....	10
Little spotted kiwi.....	12
Thesis structure.....	17
Contributions to chapters.....	19
Chapter 2: Chick Timer™ software proves an accurate, disturbance-minimizing tool for monitoring hatching success in little spotted kiwi (<i>Apteryx owenii</i>)	21
Abstract	21
Introduction	22
Materials and methods.....	24
Study site	24
Radio tags and software.....	24
Ground-truthing data	25
Activity	25
Emergence time	25
Incubation and hatching signals	26
Statistical analyses.....	26
Results.....	26
Activity	26
Emergence time	28
Predicting incubation and hatching	29
Discussion.....	29
Activity	29
Emergence time	30
Incubation events.....	30

Hatching events	31
Applications and challenges	31
Appendix 2A – How NiB Chick Timer™ tags record activity	33
Appendix 2B – Decoding an NiB Chick Timer™ tag output.....	34
Chapter 3: Population growth is not a suitable measure of translocation success: reduced hatching success suggests cryptic inbreeding depression in a growing translocated population of little spotted kiwi.....	34
Abstract.....	35
Introduction.....	36
Materials and methods	38
Study species	38
Study sites	38
Monitoring LSK reproduction and behaviour.....	38
Climate	40
Statistical analyses	40
Results	41
Reproductive effort.....	41
Hatching success	44
Climate	44
Timing of nesting	45
Incubation activity	45
Causes of egg failure.....	46
Discussion	47
Appendix 3A	53
Chapter 4: Valid estimates of individual inbreeding coefficients from marker- based pedigrees are not feasible in wild populations with low allelic diversity	57
Abstract.....	57
Introduction.....	58
Methods	61
The simulation model.....	61
Population scenarios.....	61
Sampling regimes	64
Pedigree reconstructions.....	64

Statistical analyses.....	65
Results.....	66
COLONY performance.....	66
Accuracy of parentage and full sibling assignments	67
Validity of RF _{PED}	69
Discussion.....	73
Accuracy of parent-offspring and full sibling assignments.....	73
Validity of RF _{PED}	74
Implications	76
Appendix 4A.....	79
Appendix 4B.....	84
Chapter 5: Inter-generational genetic erosion and extreme inbreeding depression in an introduced population of little spotted kiwi.....	85
Abstract	85
Introduction	86
Methods.....	89
Study sites.....	89
Sample collection and genotyping	90
Defining filial groups	91
Measuring genetic diversity.....	91
Assessing inbreeding and inbreeding avoidance.....	92
Testing for inbreeding depression.....	93
Results.....	95
Population demography	95
Genetic erosion.....	97
Inbreeding and inbreeding avoidance	98
Inbreeding depression.....	101
Discussion.....	102
Genetic erosion between filial groups.....	102
Inbreeding and inbreeding avoidance	103
Inbreeding depression on Long Island	105
Conclusion.....	107
Appendix 5A.....	109

Chapter 6: General discussion	115
Chapter overviews.....	116
Conclusion and limitations: Is there evidence of inbreeding depression in LSK?	118
Conservation implications.....	120
Management recommendations and future research	125
Monitoring of LSK.....	126
Future translocations of LSK.....	128
The conservation value of Long Island LSK	129
Summary	131
Literature cited	133

Chapter 1: Introduction

It is widely acknowledged that our planet is in the midst of a sixth mass extinction, with human-induced extinction rates up to 1,000 times the natural rate (Pimm et al. 1995). As a result, a great deal of conservation research has been focussed on factors that influence extinction risk. Population viability analyses (PVAs) have become a popular method of determining risk factors and likely chances of persistence for threatened species (Beissinger 2002). The results of such analyses can be used both to mitigate the risks of extinction and to prioritise conservation actions based on their likelihood of success. Assessing the viability of a population under different scenarios requires a large amount of data on the species in question, including information on life history, population demography and genetics (Lacy 1993). The last of these, population genetics, has often been overlooked in PVA, but is now recognized as a crucial factor in determining long-term viability (Allendorf & Ryman 2002; Frankham 2010).

Rapid reductions in population sizes of wild species frequently lead to population bottlenecks that reduce genetic diversity and increase the risk of inbreeding and inbreeding depression (Allendorf & Luikart 2007). Managing threatened species via

translocations of a few individuals to suitable habitat also causes bottlenecks that exacerbate genetic erosion and promote further inbreeding (Groombridge et al. 2012; Jamieson & Lacy 2012). Low genetic diversity and inbreeding depression are known to influence extinction risk, yet they are often ignored when assigning endangerment status or designing management plans (Jamieson et al. 2006; Frankham 2010). This is in part due to other threats, such as introduced predators or habitat loss, being seen as more pressing and in part due to the difficulty of studying population genetics in many threatened species. Experiencing a bottleneck certainly does not condemn a species to extinction (Milot et al. 2007; Johnson et al. 2009). However, testing for inbreeding depression in species that are known to have experienced recent, severe bottlenecks is crucial to designing appropriate management strategies that will ensure long-term viability. Detecting inbreeding depression requires measures of fitness and error-free measures of individual inbreeding, both of which can be challenging to obtain for wild populations. In this study, I address these challenges in a species that is in apparent recovery from a recent and severe population bottleneck, New Zealand's little spotted kiwi (*Apteryx owenii*) (LSK).

Bottleneck to extinction vortex – the role of genetics in extinction

When a species or population experiences a bottleneck, three things happen that can increase the likelihood of extinction (Fig. 1.1). First, the post-bottleneck population will have lower heterozygosity and lower allelic diversity than the pre-bottleneck population due to genetic drift (Fig. 1.2). Lower genetic diversity makes it difficult for species to respond to novel environmental challenges (Willi et al. 2006). Second, the post-bottleneck population will also be more at risk of inbreeding due to the effect of small population size (Crow & Kimura 1970). Inbreeding further erodes genetic diversity, increasing homozygosity (Wright 1977) and thus decreasing heterozygosity. This results in two negative effects: increased homozygosity of rare, deleterious alleles and decreased heterozygosity at loci where heterozygotes have an advantage (such as the major histocompatibility complex) (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009). Both these phenomena lead to a decrease in the fitness of the progeny of related individuals versus those of unrelated individuals, commonly termed inbreeding depression (the third major

outcome of a bottleneck). The increased expression of deleterious alleles is thought to be a stronger driver of inbreeding depression than decreased heterozygosity (although the effects of each can be difficult to tease apart) (Charlesworth & Charlesworth 1987; Keller & Waller 2002). Inbreeding depression is not a new concept (Darwin 1868; Ives & Whitlock 2002), but there has been much debate regarding its importance, and that of genetics in general, in population extinction risk.

Figure 1.1. The three main outcomes of a bottleneck that can push a species or population towards an extinction vortex (labelled 1, 2 and 3) and the interaction between these factors.

Population bottlenecks are often argued to be unimportant to a species' likelihood of persistence based on examples of species that have survived bottlenecks seemingly unscathed (Caro & Laurenson 1994; Craig 1994). The Chatham Island black robin (*Petroica traversi*) (Butler & Merton 1992) and the Mauritius kestrel (*Falco punctatus*) (Hurvich & Tsai 1989) have both survived extreme bottlenecks of two to three individuals. Other species, such as the wandering and Amsterdam albatrosses (*Diomedea exulans* and *D.amsterdamensis*) (Milot et al. 2007) and the Madagascar fish eagle (*Haliaeetus vociferoides*) (Johnson et al. 2009) have persisted for many generations with low genetic diversity. However, these examples are likely the exceptions rather than the rule. Focusing on species that have survived bottlenecks

ignores unobserved populations that have already become extinct following bottlenecks (Soulé 1987). This is akin to stating that smoking is not harmful because some smokers survive to old age (Allendorf & Ryman 2002).

Figure 1.2. Loss of genetic diversity following a population bottleneck.

It has also been argued that inbreeding does not impact wild populations because species are driven to extinction by non-genetic factors before genetic factors could impact them (Lande 1988; Young 1991; Caughley 1994; Craig 1994; Elgar & Clode 2001). Deleterious traits correlated with inbreeding have now been demonstrated in numerous characteristics over a variety of life history stages in wild populations. Examples include: increased developmental asymmetry and lowered tolerance to hypoxia in topminnow (*Poeciliopsis monacha*) (Vrijenhoek & Lerman 1982; Vrijenhoek et al. 1992); lower litter size and increased frequency of abnormal offspring in adders (*Vipera berus*) (Madsen et al. 1996); decreased sperm competitiveness in flour beetles (*Tribolium castaneum*) (Michalczyk et al. 2010); decreased incidence of twins in moose (*Alces alces*) (Haanes et al. 2013); and decreased survival probabilities of offspring in European bison (*Bison bonasus*) (Tokarska et al. 2011), red deer (*Cervus elaphus*) (Walling et al. 2011) and yellow-bellied marmots (*Marmota flaviventris*) (Olson et al. 2012). The impact of inbreeding depression over a variety of traits means that it can be problematic to

detect unless a whole series of life history stages are measured (Grueber et al. 2010). However, when inbreeding strongly affects embryo survival, measuring fitness at later life stages will fail to detect inbreeding depression (Hemmings et al. 2012). Due to the difficulty of obtaining long-term life history data (especially in long-lived species), the majority of inbreeding depression studies have focused on juvenile traits (Crnokrak & Roff 1999).

A final argument against the importance of inbreeding to conservation has been that inbreeding depression will result in the purging of deleterious alleles (Templeton & Read 1984; Crnokrak & Barrett 2002), effectively increasing the fitness of inbred populations. However, while lethal alleles can be purged in this way, deleterious alleles of small effect would still drift to fixation within an inbred population (Hedrick 1994). Fixation of deleterious alleles can result in an apparent lack of inbreeding depression due to depressed fitness of outbred individuals rather than increased fitness of inbred individuals (Byers & Waller 1999). Over time, this decreases overall fitness within populations, making extinction more likely (Lynch et al. 1995; Jamieson et al. 2006). It has been shown that purging is not an effective eliminator of inbreeding depression (Leberg & Firmin 2008), even in populations that provide ideal conditions for purging to occur (Kennedy et al. 2013).

It is now acknowledged that inbreeding depression can contribute significantly to population extinction risk (Frankham 1995; Keller & Waller 2002; Frankham 2005; O'Grady et al. 2006), and that this is likely to be more apparent in the face of environmental challenges (O'Brien 2000; Keller et al. 2002; Reed et al. 2002; Armbruster & Reed 2005). There have been repeated calls for genetic measures, including inbreeding depression, to be incorporated into conservation management and PVAs (Allendorf & Ryman 2002; Jamieson et al. 2006; Frankham 2010). Though desirable for conservation, measuring inbreeding in wild populations can be problematic.

Measuring inbreeding

The detection of inbreeding depression on an individual basis is reliant on accurate measurement of individual inbreeding coefficients. The inbreeding coefficient (F) was first suggested by Wright (1921) as the correlation between homologous genes of two gametes, but was reinterpreted by Malécot (1948) as the probability of portions of the genome being identical by descent (IBD) (i.e., the probability they were inherited from a common ancestor). F ranges from zero (completely outbred, no portion of the genome IBD) to one (completely inbred, entire genome IBD). Errors in the measurement of inbreeding can lead to underestimation of inbreeding depression (Reid et al. 2013), which, in turn, could result in the mismanagement of populations. There are three main tools currently available to researchers wishing to estimate F : pedigrees, multilocus heterozygosity and direct marker-based estimates. Each method has problems and pitfalls.

Pedigrees are frequently recommended as the best way to measure individual inbreeding coefficients (Pemberton 2004; Slate et al. 2004; Pemberton 2008; Jones & Wang 2009; Taylor et al. 2010; Grueber et al. 2011). The pedigree inbreeding coefficient (F_{PED}) is calculated by tracing the ancestry of an individual through an established pedigree, often via path analysis (Wright 1922). This results in an estimate of F relative to a baseline population, usually defined as the founders of the pedigree, who are assumed to be unrelated and non-inbred (Keller & Waller 2002). It is unlikely, however, that founders will ever be completely unrelated; all individuals in a species are related if their ancestry is traced back far enough. Ignoring this fact leads to underestimation of inbreeding coefficients (Jones et al. 2002; Russello & Amato 2004). In addition, F_{PED} does not account for the variance in IBD between individuals with identical F_{PED} that occurs due to chance (Hill & Weir 2011; Forstmeier et al. 2012), making it a frequently inaccurate estimator of F . Finally, the long-term behavioural data required to construct accurate pedigrees is rarely available for wild populations and can be confounded by undetected extra-pair fertilizations (Pemberton 2008; Jones & Wang 2009), resulting in shallow and/or inaccurate pedigrees that provide invalid estimates of F .

Inbreeding depression can alternatively be detected via correlations between fitness traits and individual multilocus heterozygosity (heterozygosity fitness correlations – HFCs), or estimating relatedness and/or inbreeding coefficients directly using genetic markers. HFCs have been repeatedly criticised due to their absence in large, outbred populations (Balloux et al. 2004; Pemberton 2004; Slate et al. 2004), their failure to predict inbreeding in small, bottlenecked populations (Grueber et al. 2008; Grueber et al. 2011) and the discussion of whether HFCs are attributable to inbreeding (general effect) rather than local effects caused by linkage or direct effects caused by selection at genotyped loci (Hansson & Westerberg 2002). The main issue with HFCs as they are currently used is that the microsatellites they are normally based on are known to be relatively poor estimators of genome-wide diversity (Slate et al. 2004; DeWoody & DeWoody 2005) and so, without unfeasibly large numbers of microsatellites and/or high levels of allelic diversity, detecting HFCs is often impossible, even when there is high variance in inbreeding (Grueber et al. 2011). This can be remedied by using large panels of single nucleotide polymorphisms (SNPs) (Hoffman et al. 2014b), although these are not yet readily available for the majority of non-model species.

Relatedness and inbreeding coefficients can also be estimated directly from genetic markers using either moment estimators (that estimate the relatedness between individuals in terms of probabilities of identity by descent) (Queller & Goodnight 1989; Li et al. 1993; Ritland 1996; Lynch & Ritland 1999; Wang 2002) or likelihood methods (that calculate the probability of individuals falling into a particular relationship given the marker data available) (Anderson & Weir 2007; Wang 2007). No one estimator performs best in all scenarios and, again, these methods are affected by the number and diversity of loci available (Van de Casteele et al. 2001; Wang 2011). Even when sufficient markers are available, estimates are dependent on the reference population used in the analysis (Wang 2014) (an issue analogous to that of assuming unrelated founders in a pedigree), and the reliability of the estimates produced by these techniques is debatable, especially for populations that have experienced genetic bottlenecks (Robinson et al. 2013). Thus, in spite of the issues inherent in using pedigrees to measure inbreeding, they are still often seen as the best tool available (Santure et al. 2010).

One of the biggest challenges in using pedigrees to measure inbreeding is arguably the lack of behavioural data available for their construction. Behaviour-based pedigrees can be supplemented with genetic data from individual marker-based genotypes (e.g., Gelatt et al. 2010; Goncalves da Silva et al. 2010). In some cases, pedigrees can be constructed using genetic markers alone (Koch et al. 2008; Wang & Santure 2009; Mourier et al. 2013) and several software programs implementing maximum likelihood algorithms have been developed to facilitate this (Marshall et al. 1998; Gerber et al. 2003; Jones & Wang 2010). The accuracy of marker-based pedigrees is known to vary with the number and allelic diversity of loci available, completeness of population sampling, and genotyping error (Estoup et al. 1998; Marshall et al. 1998; Bernatchez & Duchesne 2000; Nielsen et al. 2001; Koch et al. 2008; Harrison et al. 2013). In threatened species, genetic diversity is often low, reducing the power of markers available to construct pedigrees. The feasibility of constructing accurate pedigrees using only genetic markers in threatened species and the effect of pedigree inaccuracy on the validity of pedigree inbreeding coefficients in such scenarios has not yet been specifically examined.

Microsatellite markers for conservation genetics

This study, like the majority of conservation genetics research, uses microsatellite markers to make inferences regarding population genetics. While single nucleotide polymorphisms (SNPs) and whole genome sequencing (WGS) are becoming increasingly common in population genetics in general (Schlotterer 2004; Allendorf et al. 2013; Ekblom & Wolf 2014), the majority of conservation geneticists working on non-model species do not currently have access to these tools and thus continue to use microsatellite markers (Schlotterer 2004; Guichoux et al. 2011).

Microsatellite markers remain popular with good reason; their relatively rapid mutation rate results in high variability, which increases their power for use in population genetic tests. Microsatellites can also be highly cost effective, as markers developed in one species will often cross-amplify in closely related species, reducing the need for discovering new markers for each species in a series of studies. There are, however, some disadvantages and caveats to using this type of marker that

warrant discussion. Here, I highlight the main issues. For a full review on effective implementation of microsatellites in ecological and conservation studies, please refer to Selkoe and Toonen (2006).

Accurate allele-calling (or scoring) is arguably the most important factor when using microsatellites. Rerunning a subset of individuals at each locus used is recommended to allow estimation of average genotyping error for a given locus or set of loci (Pompanon et al. 2005) and loci that do not amplify reliably should be excluded from further analysis. Null alleles are a less readily detectable source of error that stem from certain alleles at a locus not producing a PCR product due to poor primer binding (Callen et al. 1993). An individual that is heterozygous for a normal and a null allele will appear homozygous for the normal allele thus null alleles typically result in an excess of homozygotes compared to that predicted by Hardy-Weinberg equilibrium (Brookfield 1996). Programs such as MICRO-CHECKER (Van Oosterhout et al. 2004) can be used to screen loci for null alleles.

Homoplasy can also lead to scoring error and involves alleles that appear identical in their size-state on an electropherogram, but are not identical by descent (Estoup et al. 2002). Homoplasy can be divided into two types, termed by Selkoe and Toonen (2006) as “undetectable” and “detectable” homoplasy. Undetectable homoplasy involves alleles with exactly the same sequence that have evolved via different pathways (e.g., via a “back-mutation” to a previous state) (Selkoe & Toonen 2006). Detectable homoplasy refers to alleles of the same size, but with differences in their sequence caused by point mutations or insertions/deletions in the flanking region of the marker (Grimaldi & CrouauRoy 1997). The latter form can, as the name suggests, be detected by sequencing the same allele in a number of individuals or using the single strand conformational polymorphism method (Angers et al. 2000; Sunnucks et al. 2000), but the former kind is almost impossible to identify. Undetected homoplasy can lead to an underestimation of the genetic divergence between populations. However, homoplasy is usually compensated for by the high variability of microsatellite markers and is not considered a significant issue for the majority of population genetics tests implemented by molecular ecologists (Estoup et al. 2002).

The fact that microsatellites are generally discovered in one species and then used in other, closely related species can lead to a phenomenon called ascertainment bias. Here, markers are targeted on the basis of being highly polymorphic in the focal species, but homologous loci may be less variable in sister species (Ellegren et al. 1997). This makes it difficult to compare genetic variation across species using the same marker set. Interestingly, genetic variation in little spotted kiwi (LSK) is so low that, when 14 microsatellites discovered in this species were tested in the other four species of kiwi, the markers were more, rather than less, variable in all the other species in almost every instance (Ramstad et al. 2010). Thus, ascertainment bias does not appear to be an issue for LSK.

Routine screening of microsatellite data sets should also include checks for gametic disequilibrium (linkage of loci that results in pseudoreplication) (Allendorf et al. 2013) and whether loci appear to be under selection rather than selectively neutral (usually due to linkage with a locus that is under selection) (Ford 2002). A variety of programs, including GenAlEx (Peakall & Smouse 2006, 2012) and Genepop (Raymond & Rousset 1995) allow such tests to be run.

Conservation translocations

Due to habitat loss and invasive predators and pests, translocations of individuals to found populations of threatened species in new or previously occupied areas of suitable habitat are an increasingly prevalent conservation tool (Seddon 2010). In general, translocations have produced mixed results (Wolf et al. 1998; Fischer & Lindenmayer 2000; Seddon et al. 2012; Miller et al. 2014). The Arabian oryx (*Oryx leucoryx*) (Spalton et al. 1999), golden lion tamarin (*Leontopithecus rosalia*) (Russo 2009) and large blue butterfly (*Phengaris arion*) (Thomas et al. 2009) are evidence of the potential for successful translocations, but there have also been many failed translocation attempts (Godefroid et al. 2011; Kingsley et al. 2012; Bennett et al. 2013; Lintermans 2013). The causes of failure for many translocations have been left unrecorded due to a lack of post-introduction monitoring (Fischer & Lindenmayer 2000). As a result, the importance of short and long-term post-translocation monitoring to allow for adaptive management and the improvement of

translocation practices has been emphasised repeatedly (Lyles & May 1987; Hein 1997; Ewen & Armstrong 2007; IUCN/SSC 2013).

For many translocations, post-introduction monitoring takes the form of measuring population growth, as this is considered a “catch all” for several vital rates (Nichols & Armstrong 2012). However, it is recommended that specific vital rates such as reproduction and survival are also monitored individually in the years following a translocation event (IUCN/SSC 2013). Reproductive success in particular is one of the key factors in modelling long term viability in translocated populations (Armstrong et al. 2002). Reproductive output of any population is known to be affected by numerous factors including (but not limited to) climate (Ismail et al. 2011), social interactions (Lopez-Sepulcre et al. 2009), habitat conditions (Dalbeck & Heg 2006) and genetics (Briskie & Mackintosh 2004). In translocated populations, food availability and inbreeding depression in particular can impact on reproductive success. Translocation sites are normally chosen to be favourable for the species being moved; in New Zealand, this normally equates to predator-free island or fenced sanctuary sites with limited dispersal opportunities. Thus translocated populations (particularly birds) may grow to outstrip their food supply (Mackintosh & Briskie 2005). Additionally, translocated populations founded with few individuals are more likely to be inbred due to the effects of small population size (Crow & Kimura 1970), especially when they exist in closed, island-style populations with no migration and therefore no gene flow. Even when larger numbers of founders are translocated, reproductive skew in those founders could still lead to genetic erosion and inbreeding (Anthony & Blumstein 2000; Miller et al. 2009). Decreased reproductive success in a translocated population might indicate inbreeding depression, but genetic, as well as ecological monitoring is necessary to confirm this.

Genetic factors have been frequently ignored in translocation monitoring due to the fact that they act on a longer timescale than more immediate concerns such as habitat loss and introduced predators (Seddon et al. 2007; Jamieson et al. 2008). Loss of genetic diversity and inbreeding depression following translocations can be mitigated by using sufficient numbers of founders (Miller et al. 2009), but

introductions and reintroductions often involve small numbers of individuals for pragmatic reasons (Jamieson & Lacy 2012). Thus, translocated populations are very much at risk of genetic erosion and inbreeding depression. The genetic diversity and relatedness of potential founders are rarely assessed due to logistical and financial constraints (Groombridge et al. 2012) (although see Haig et al. 1990; Nader et al. 1999; Rocamora & Richardson 2003; Hansson & Richardson 2005a; and Zhang et al. 2006 for exceptions to this), making post-translocation genetic monitoring crucial to understanding underlying factors that may be impacting the success of the translocation. Several studies have noted a loss of genetic diversity and increased inbreeding following conservation introductions and reintroductions (e.g., Ellegren et al. 1993; Gautschi et al. 2003; Brekke et al. 2010; Brekke et al. 2011) and the severity of a bottleneck during translocations is known to impact their likelihood of failure (Thevenon & Couvet 2002). Long-term genetic monitoring allows for genetic management of populations via supplementary translocations of genetically distinct individuals (Groombridge et al. 2012; Heber et al. 2013). While it is important to view the role of genetics in translocations in the context of other, more immediate threats (Jamieson & Lacy 2012), there is no doubt that incorporating long-term genetic monitoring into translocation management would improve the long-term success of translocations. This is particularly true in countries such as New Zealand, where a large number of species have experienced population bottlenecks (Briskie & Mackintosh 2004) and where translocations to predator-free islands and fenced sanctuaries have been used extensively for conservation management (Saunders 1994).

Little spotted kiwi

The little spotted kiwi (LSK) (Fig. 1.3) is the smallest and second rarest of New Zealand's five endemic kiwi species (Heather & Robertson 2005; Holzapfel et al. 2008). Along with the other four species of kiwi, LSK have always had special significance to the Māori (the indigenous people of New Zealand) and are a cultural icon to all New Zealanders (Holzapfel et al. 2008). LSK are flightless, nocturnal and insectivorous. They are also long-lived (life expectancy ranges from 27-83 years in the wild) (Robertson & Colbourne 2004) and socially monogamous (Heather & Robertson 2005). Female LSK produce one of the largest eggs relative to body size

of any bird (Calder et al. 1978), but it is the male who is solely responsible for incubation, which can last for 65-75 days. Nesting occurs in subterranean burrows and, if successful, results in precocial chicks that remain in the nest with the male for several weeks, but receive no detectable parental care (Heather & Robertson 2005).

Figure 1.3. A little spotted kiwi in its burrow.

LSK provide an excellent opportunity to examine inbreeding depression in a wild species with a history of bottlenecks and translocations. This species was historically widespread throughout mainland New Zealand, but like much of New Zealand's avifauna, was decimated by introduced mammalian predators associated with the arrival of Polynesian and European settlers. This, coupled with a profitable trade in LSK skins for muffs and museum specimens in Europe during early European settlement of New Zealand, led to the extirpation of the mainland LSK population by the 1980s (Worthy & Holdaway 2002; Holzapfel et al. 2008). The species persisted on Kapiti Island, a seemingly successful population off the west coast of New Zealand's North Island and on D'Urville Island in the Marlborough Sounds through a handful of remnant birds (Fig. 1.4). The Kapiti Island population was founded by, at most, five birds that were translocated to Kapiti Island from the South Island of New Zealand in 1912 (Ramstad et al. 2013). This population has grown rapidly in the past 100 years, and numbered ~1,200 birds in 2005 (Heather & Robertson 2005). As a result, the New Zealand Department of Conservation has

been able to found seven new LSK populations on six off-shore predator-free islands and in one mainland predator-free sanctuary via the translocation of Kapiti Island birds (Fig. 1.4) (Colbourne & Robertson 1997, R. Colbourne, unpublished). LSK currently number ~1,700 birds (H. Robertson, unpublished).

Figure 1.4. Map of New Zealand showing the location of the eight extant LSK populations (blue dots) and two extinct source populations. Kapiti Island is the source population for all seven other extant populations, for which number of founders and date(s) of founder translocation(s) are noted. Although two remnant birds from D'Urville and a third bird from Kapiti Island were translocated to Long Island, they appear to have died without reproducing.

The translocation of five birds to Kapiti Island undoubtedly saved LSK from extinction, but it also resulted in an extreme genetic bottleneck, which has left LSK with very low genetic diversity at both neutral and functional loci (Ramstad et al. 2010; Miller et al. 2011; Ramstad et al. 2013). The subsequent translocation of birds from Kapiti Island to found new populations has caused secondary bottlenecks of between two and 40 individuals (Fig. 1.4) and the loss of further heterozygosity and allelic diversity to varying extents in the three recently translocated populations that

have been genetically surveyed (Ramstad et al. 2013). The success of LSK conservation has largely been monitored via population growth, which has been positive in all eight LSK populations since their founding. As a result of this, the IUCN recently downgraded LSK from “Vulnerable” to “Near Threatened” (Birdlife International 2013); it is the only species of kiwi not considered threatened in the latest New Zealand bird conservation listing, which classed it as “in recovery” (Miskelly et al. 2008). These endangerment ratings do not take into account the risks posed by the incredibly low genetic diversity exhibited by this species. With the exception of one study on reproductive success of Kapiti Island LSK in the late 1980s (Jolly 1989), no specific measures of vital rates have ever been taken for any LSK population. There is potential for inbreeding in some of the recently translocated populations of LSK due to low founder numbers (Fig. 1.4), but inbreeding depression has never been investigated in this species. No pedigree information is available for LSK except the identities of the founders for the seven recent translocations. Here, I characterise the hatching success and genetics of the two LSK populations with the most (Zealandia) and least (Long Island) founders and test for inbreeding depression in each.

Zealandia Sanctuary (Fig. 1.4 and Fig. 1.5a) is a 225ha mainland island sanctuary in Wellington, New Zealand, consisting of native podocarp and introduced pine forest on a steep sided valley. The sanctuary is surrounded by a 2.2m high fence that is impervious to the majority of introduced mammalian predators. LSK were introduced to Zealandia from Kapiti Island in 2000 (eight females and 12 males) and 2001 (10 females and 10 males). The LSK population in Zealandia has shown 9.5% growth per year since these introductions and the estimated population size is currently ~120 individuals (H.Robertson pers. comm., June 2012). Genetic diversity of Zealandia LSK has not been characterized to date.

Figure 1.5. a) Zealandia Sanctuary in the Karori Valley is on the outskirts of New Zealand's capital city, Wellington. It is a 225ha predator free sanctuary completely enclosed by a predator proof fence. b) Long Island in the Marlborough Sounds, at the northern end of New Zealand's South Island, is a 160ha land ridge that was cleared of mammalian predators in 1992.

Long Island (Fig. 1.4 and Fig. 1.5b) is a 160ha ridge of land at the northern end of the Queen Charlotte Sound in Marlborough, New Zealand. The island was cleared of mammalian predators in 1992 although an avian kiwi nest predator, the weka (*Gallirallus australis*), is occasionally present on the island. Two male Kapiti Island LSK were introduced to Long Island in 1982 along with a female LSK that had been discovered on D'Urville Island. A male LSK was also found on D'Urville and translocated to Long Island in 1987. All other D'Urville Island birds are since thought to have perished. In 1989, one of the Kapiti Island males was removed from Long Island and a Kapiti Island female was introduced, leaving two Kapiti Island birds and the last two known D'Urville Island birds (Colbourne & Robertson 1997; Jolly & Daugherty 2002). The D'Urville birds had distinct genotypes when compared to Kapiti Island LSK and had been put on Long Island with the hope they would breed and produce birds that could introduce genetic variation into other LSK populations. Unfortunately, genetic surveys of Long Island LSK have failed to detect any genetic material from the D'Urville birds among LSK hatched on Long Island. Thus, the D'Urville birds seem to have died without producing descendants (Ramstad et al. 2013) and the entire Long Island population is thought to be descended from the two Kapiti Island founders, who are still alive and producing

chicks. This represents a severe secondary bottleneck for Long Island LSK and, consequently, genetic diversity on Long Island is the lowest of any LSK population yet studied (Ramstad et al. 2013). In spite of this, the Long Island population has grown by 11% per year since its foundation and is currently estimated to number ~50 individuals (Robertson & Colbourne 2008, H. Robertson, unpublished).

Minimizing further loss of genetic diversity is a priority for all kiwi species (Holzapfel et al. 2008) and investigating the results of the bottlenecks experienced by LSK in particular is a high priority action (Holzapfel et al. 2008). As the majority of mainland New Zealand is still overrun with introduced mammalian predators, LSK will be confined to predator-free islands and predator-proof mainland sanctuaries for the foreseeable future. Plans are underway to found new island-based LSK populations (H. Robertson, pers. comm., 7th January 2014) and to manage LSK populations as a metapopulation with human-mediated migration assisting gene flow between sites (Ramstad et al. 2013). An understanding of the likely impact of inbreeding depression on LSK populations will facilitate their future management. Investigating inbreeding in LSK also creates an opportunity to add to the growing body of literature on inbreeding depression in wild populations with data from an unrepresented taxonomic group. Finally, this study allows a thorough investigation of the performance of current tools available to measure inbreeding and detect inbreeding depression when applied to wild populations of conservation concern.

Thesis structure

This thesis comprises four research chapters that are formatted for submission to peer reviewed journals. This inevitably results in some repetition among chapters, particularly in introduction sections, but is advantageous in producing separate studies while addressing overarching research questions. The research chapters are the result of collaborative efforts. Therefore, I use the collective term “we” to reflect the contributions of all co-authors. The overarching aim of the thesis is to test for inbreeding depression in two populations of LSK. This goal was addressed via the following three questions:

1. *What is the reproductive effort and hatching success rate in Zealandia and Long Island LSK?*

I chose to monitor hatching success in LSK as a measure of reproductive success. Monitoring hatching success in cryptic and nocturnal species can be challenging. In Chapter 2 (published in NZ Journal of Zoology) (Taylor et al. 2014), I test the reliability of radio tracking software that has never been used in LSK before. Having found this to be a reliable tool, in Chapter 3 (in preparation Animal Conservation), I measure hatching success over two seasons in the Long Island and Zealandia populations of LSK.

2. *Can we reconstruct pedigrees using genetic data to measure inbreeding in LSK?*

To link hatching success to inbreeding, it is necessary to measure inbreeding in LSK. Pedigrees are frequently recommended for measuring individual inbreeding coefficients, but no pedigree data currently exists for any LSK population. In Chapter 4 (submitted to the Journal of Evolutionary Biology), I use a simulation-based approach to investigate the feasibility of reconstructing LSK pedigrees using only genetic data.

3. *Is there evidence of continued genetic erosion or inbreeding depression in Zealandia or Long Island LSK?*

Founding new populations using small numbers of individuals is expected to decrease genetic diversity relative to the source population and can lead to inbreeding depression. In Chapter 5 (in preparation for Conservation Genetics), I use microsatellite genotypes to split Zealandia and Long Island kiwi into subsets based on their generation (i.e., founders, first generation etc.). I quantify genetic erosion (loss of allelic diversity and heterozygosity) over each generation and test for inbreeding in both populations. I also use population demographic information and population simulations to quantify the strength of inbreeding depression in the Long Island population.

Chapter 6 is a general discussion of the results presented in the four data chapters of my thesis. In it, I summarise my findings and the evidence for inbreeding depression in LSK. I also discuss the wider conservation implications of my work, suggest future management actions for LSK and present opportunities for further research resulting from my thesis.

Contributions to chapters

All study design, data collection, analyses and writing were conducted by the author with assistance from the relevant supervisors with the following exceptions:

Data collection: For chapters 2, 3 and 5, field work was conducted by the author with field assistants. For chapters 4 and 5, the author was assisted in data collection by Marty Kardos at the University of Montana, who provided modified versions of pre-existing R-scripts designed for population simulations.

Writing and editing: John Wilks from Wildtech New Zealand Ltd contributed to the editing process for Chapter 2 and Marty Kardos contributed to the editing process for Chapter 4.

Chapter 2: Chick Timer™ software proves an accurate, disturbance-minimizing tool for monitoring hatching success in little spotted kiwi (*Apteryx owenii*)¹

Abstract

Measuring hatching success in birds is important for assessing population viability, but nests are often cryptic and may be abandoned if birds are disturbed during nesting. We evaluated the suitability of Wildtech New Zealand Ltd's Chick Timer™ radio telemetry software for monitoring hatching success in little spotted kiwi (*Apteryx owenii*). The software provided accurate reports of activity levels and times of nest emergence by adult birds. Eighty four percent of incubation attempts and 70% of first hatching events were detected, with no false indications of incubation or hatching. Our results suggest that this technology will facilitate studies of hatching success in little spotted kiwi and other large birds with cryptic or inaccessible nests. A ground truthing study such as this one, however, is required before full implementation of the software in any species.

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Introduction

Understanding the ecology of a species is crucial to its conservation, but collecting ecological and life history data can be time consuming and challenging. In particular, an accurate measure of reproductive success is a necessary parameter for population modeling, including population viability analyses (Allendorf et al. 2013), and reproductive success is widely used as a fitness indicator in inbreeding depression studies (Lacy et al. 1996; Brekke et al. 2010; Billing et al. 2012). In birds, hatching success is a frequently used proxy for reproductive output (Berg et al. 1992; Sebei et al. 2009; Brown et al. 2013). Measuring hatching success in the wild can be problematic due to the need to locate and monitor individuals over large geographic areas and relatively long time periods (Mayfield 1975). In species that are cryptic or nocturnal, locating a nest and determining its success can be especially difficult (Newman et al. 2009; Zangmeister et al. 2009). Measuring hatching success typically involves direct observation, making it time intensive and potentially disruptive for the individuals being monitored, which can lead to nest abandonment (Blackmer et al. 2004).

Radio telemetry can facilitate studies of reproductive success (Aldridge & Brigham 2001; Nguyen et al. 2003; Ziesemann et al. 2011). Increasingly sophisticated radio tag technology has been developed and it is now possible to use radio tags to measure heart rate, temperature (Signer et al. 2010) and, crucially, motion (Acquarone et al. 2001). New Zealand-based wildlife technology company Wildtech Ltd has adapted motion sensor technology for use in its Chick Timer™ software. This software can be loaded into radio tags that are fitted to kiwi (*Apteryx* spp.) to monitor their reproduction remotely, as well as recording data on their activity patterns (Appendices 2A and 2B). Kiwi are cryptic and nocturnal ratites that nest in sometimes fragile, subterranean cavities. Some species of kiwi camouflage the entrance to their nest cavities (Heather & Robertson 2005) and are known to abandon incubation in the event of excessive human disturbance (R. Colbourne and H. Robertson pers. comm., June 2012). Thus, early detection of nests and minimizing disturbance when monitoring kiwi nesting is vital.

To date, Chick Timer™ software has been used primarily to enable egg and chick collection for captive husbandry and reintroduction programs for kiwi (Colbourne et al. 2005; Holzapfel et al. 2008), but not for scientific studies of hatching success in wild populations. The Chick Timer™ program notifies the user when a bird is incubating, which makes it easier and safer for researchers investigating hatching success in the field to locate nests for the first time, and permits remote data collection from anywhere within range of the tag. This enables information to be collected on activity and nest emergence times of incubating parents, nest abandonments, and hatching events; together, this information can facilitate nest monitoring while minimizing disturbance. These advantages make Chick Timer™ software an attractive tool for measuring hatching success in kiwi and other cryptic and/or nocturnal species with a tendency to abandon nests when disturbed directly.

The little spotted kiwi (*A. owenii*, LSK) is the second rarest kiwi species, currently numbering approximately 1,700 birds (H. Robertson, pers. comm., June 2012). The species is restricted to eight isolated populations, and all individuals are descended from, at most, five birds translocated to Kapiti Island in the early 1900s (Ramstad et al. 2013). This bottleneck has resulted in extremely low genetic diversity in LSK (Miller et al. 2011; Ramstad et al. 2013) and understanding the impacts of this has been identified as a priority for their management (Holzapfel et al. 2008). Monitoring reproductive success in LSK is crucial to understanding their future viability.

Previous estimates of hatching success in LSK involved fitting individual males (the sole incubator in this species) with standard VHF radio tags, following them until a nest could be identified and then checking that nest during the night at regular intervals for the duration of incubation to ascertain whether or not an egg was present and whether or not it had hatched (Jolly 1989). This approach is thought to have led to nest failure in several cases due to a predatory endemic bird species, the weka (*Gallirallus australis*), being drawn to increased human activity around nest sites (Colbourne 1992) and an increased risk of abandonment by incubating males due to disturbance (R. Colbourne pers comm., June 2012). Despite its wide use in the other four species of kiwi, no variant of Chick Timer™ software has ever been

used in LSK. In this study, we trialed the North Island brown kiwi (NiB) version of the Chick Timer™ program as a tool for measuring hatching success in LSK. We specifically asked:

- 1) How accurate are the data outputs provided by NiB Chick Timer™ software for LSK?
- 2) How reliable are the hatching success measurements facilitated by NiB Chick Timer™ software?

Materials and methods

Study site

Zealandia is a 225ha mainland island sanctuary in Wellington, New Zealand consisting of native podocarp and introduced pine forest. The sanctuary is surrounded by a 2.2m high fence that is impervious to introduced mammalian predators, which are the primary agents of decline in LSK. Forty LSK were introduced to Zealandia between 2000 and 2001 and the current population is ~120 individuals (H. Robertson, pers. comm., June 2012).

Radio tags and software

A total of eight male LSK in Zealandia were fitted with V2L 152A VHF leg band radio tags (Sirtrack, Havelock North, NZ). Tags weighed 11g and were specially designed to provide maximum battery life while remaining within the acceptable size and weight limits for an LSK tag (Robertson & Colbourne 2003). Birds were initially located using trained kiwi tracking dogs. All kiwi capture, handling and tagging was conducted according to the New Zealand Department of Conservation's Kiwi Best Practice Manual (Robertson & Colbourne 2003).

Tags were preloaded with NiB Chick Timer™ V3.5 for little spotted kiwi (Wildtech Ltd, Havelock North, NZ) (Appendices 2A and 2B). Pulse output schemes were modified for use in LSK tags to provide maximum battery life, but the decision-making algorithms within the software were not changed. Birds were located and tag data collected in Zealandia using a Telonics TR4 receiver (Telonics, Mesa, Arizona, USA) and Yagi folding antenna (Kiwitrack, Havelock North, NZ).

Ground-truthing data

Four types of output were selected to test the accuracy of the NiB Chick Timer™ software in LSK; minutes of activity, emergence time, occurrence of incubation and occurrence of hatching.

Activity

Activity recorded by the NiB Chick Timer™ software forms the basis of incubation status, hatching, emergence time and the trigger of the desertion alert as reported by the program (Appendix 2A) and therefore it was crucial to test the accuracy of this metric. Bushnell Trophy Cam - Model 11-9436c trail cameras (Bushnell, Kansas City, USA) were positioned between one and three meters from nest entrances of five birds during November 2011-January 2012 (LSK nesting season) and used to record emergence and return times, allowing time away from the nest to be calculated. Tag data were collected for these five birds for five to 13 days per bird and video footage was downloaded from the cameras once a week. The corresponding video data and tag output data were then compared.

Emergence time

The time a bird actually exited the burrow or nest was monitored in two discrete time periods. During July 2011 (austral winter – outside of main LSK nesting season), four male birds fitted with NiB Chick Timer™ programmed tags were located every day for two weeks using radio telemetry. When the bird was found sleeping in a suitable burrow (i.e., one where the entrance could be seen clearly by an observer at night), an observer would return to that burrow before dusk, wait for the bird to emerge and record the time of emergence. The same bird was then tracked again the following day and the tag output recorded so that the corresponding time of emergence estimated by the software was also logged. Emergence time was recorded in this way five times for each of the four birds. A second set of observed exit times were collected for four additional birds as well as one of the previous birds, using the trail cameras set up at nest entrances as detailed above. NiB Chick Timer™ data were collected on five to 14 days per bird from the radio tags on these birds and then matched up with the corresponding camera data.

Incubation and hatching signals

Each tag signal of incubation initiation (N=13) and hatching (N=15) was verified by direct observation. Suspected nests were visited once the male had exited for the night and a Taupe infra-red burrowscope (Sextant Technologies, Wellington, NZ) was used to confirm the presence of an egg. To verify hatching events, nests were staked out from before dusk as soon as possible after the hatching event was indicated by the software so that either the emergence of a chick could be observed or, once the male had left, the nest could be scoped to assess chick or egg presence or absence.

Statistical analyses

Analyses were carried out and figures produced in R (R Development Core Team 2013). The alpha value for all statistical tests was set at 0.05. All data was tested for normality using a Shapiro-Wilks test.

Results

Activity

Tag-recorded activity values were paired with direct observations of birds entering and exiting the nest five to 14 times per bird (Table 2.1). The average difference in activity recorded by the software and that observed on the cameras ranged from 2-17 minutes between birds with the average difference across birds being three minutes (Fig. 2.1A). Significant differences between tag and camera data were observed in two of the five birds, but only five software outputs spread across three different tags showed an error of more than 30 minutes (Table 2.1). The majority of differences were positive (Fig. 2.1A), indicating that the software was more likely to report longer periods of activity than those observed based on camera-recorded nest exit and return times.

Table 2.1. Mean activity and emergence times recorded by NiB Chick Timer™ software (CT) and collected via direct observation for male LSK in Zealandia Sanctuary. N is the number of observations per bird.

Bird ID	Time active (mins)				Hours since emergence			
	n	CT (mean ± 95% CIs)	Observed (mean ± 95% CIs)	Paired t-test	n	CT (mean ± 95% CIs)	Observed (mean ± 95% CIs)	Paired t-test
1	-	-	-	-	5	19.40 ± 2.81	19.20 ± 3.00	t(4) = 0.30, P = 0.78
2	-	-	-	-	5	19.00 ± 1.86	18.00 ± 1.86	t(4) = 8.69, P<0.001
3	-	-	-	-	5	20.40 ± 2.37	19.80 ± 2.18	t(4) = 4.91, P<0.01
4	5	452.00 ± 31.85	462.20 ± 28.20	t(4) = -2.27, P = 0.09	11	17.00 ± 1.50	16.30 ± 1.52	t(10) = 8.09, P<0.001
5	13	340.00 ± 43.60	322.62 ± 45.14	t(12) = 5.85, P<0.001	14	15.07 ± 1.29	14.21 ± 1.27	t(13) = 8.76, P<0.001
6	13	296.92 ± 48.18	296.92 ± 49.27	t(12) = 0.86, P = 0.40	13	15.23 ± 1.18	14.69 ± 1.28	t(12) = 4.45, P<0.001
7	11	267.27 ± 34.77	269.73 ± 34.26	t(10) = -0.31, P = 0.76	12	14.40 ± 1.16	13.80 ± 1.16	t(11) = 7.68, P<0.001
8	8	471.25 ± 55.24	457.88 ± 63.11	t(7) = 2.43, P = 0.05	5	16.80 ± 1.57	16.00 ± 1.86	t(4) = 6.71, P<0.01

Figure 2.1. Difference in (A) activity and (B) emergence time recorded by the NiB Chick Timer™ software and collected via direct observation (data are pooled across all birds tested). In A, positive values indicate that the software recorded a longer period of activity than that observed directly and negative values indicate that the software recorded a shorter period of activity than that observed directly (n=50 observation across 5 birds). In B, positive values indicate that the software recorded an earlier emergence time than that observed directly and negative values indicate that the software recorded a later emergence time than that observed directly (n=68 observations across 8 birds). In both plots, the boxes represent the upper and lower quartiles, divided by the median. The 10 and 90 percent quartiles are depicted by lines and dots represent the outliers.

Emergence time

Paired data for emergence times recorded from tags and direct observations of bird emergence time varied from 5-14 observations per bird (Table 2.1). The software consistently recorded an earlier emergence time than that actually observed (Fig. 2.1B) and significant differences between tag data and that from direct observation were recorded in all birds except bird 1 (Table 2.1). The mean difference between recorded and observed emergence time for each tag was never more than one hour and the majority (83%) of software outputs fell within an error of one hour from the directly observed time (Fig. 2.1B).

Predicting incubation and hatching

The NiB Chick Timer™ software detected 85% (11/13) of incubation attempts made by male LSK. The software also registered 80% (4/5) of hatches in single egg nests, 60% (3/5) of first hatches in two-egg nests and 60% (3/5) of second egg hatches in two-egg nests. In two cases the tag missed the first hatching event in a clutch and recorded the second. Throughout the 2011-2012 field season, there were no false indications of incubation or hatching by the tag software.

Discussion

Hatching success is an important measure of reproductive success across bird species, but measuring hatching success in the wild remains problematic (Blackmer et al. 2004; Carey 2009, 2011). In this study, we tested the accuracy of the NiB Chick Timer™ software for measuring hatching success in LSK. We found the program to be an effective and highly suitable tool, although there are some caveats to its wider implementation.

Activity

All data outputs from Chick Timer™ software are based on the activity recorded by the tag thus it is crucial that activity is measured accurately. Our results suggest the activity measured and reported by the software is very close to the birds' actual activity. Significant differences between tag activity reports and direct observation data were found in two birds. However, the majority of differences were less than 30 minutes and the largest difference for any tag on any night was 74 minutes. As the difference in activity levels between an incubating and non-incubating LSK male is, on average, ~300 minutes per night (Chapter 3), this level of error would not lead the software to give a false indication of incubation or, conversely, to miss an incubation event.

Reporting a longer period of activity than that observed could be due to birds moving around inside the nest before exit and/or after their return. The degree to which this occurs would depend on individual behaviour and space available within individual nests, leading to differences in accuracy between birds. Conversely,

reporting shorter periods of activity than that indicated by nest exit and return times could be caused by the birds resting while away from the nest, reflecting the limitations of emergence and re-entry times as a proxy for activity levels.

Emergence time

In kiwi management, emergence time is frequently used to plan researcher arrival time at burrows and nests for the purpose of catching males and chicks (especially during nesting season when emergence times can be erratic). As a result, Chick Timer™ software is designed to record an emergence time slightly earlier than that actually occurring to avoid missing a bird exiting its burrow. In addition, Chick Timer™ software is only able to count time since emergence in hour intervals, leading to imprecise estimates. Thus, the statistically significant differences seen between tag-reported and directly observed emergence times across all but one bird in this study is accounted for as an artefact of the software design. As no one tag showed a mean difference of more than an hour and estimates were almost always earlier than the real emergence time, the differences are, again, statistically, but not practically significant.

Incubation events

There were two instances where the software failed to register an incubation event. In both cases, the bird did not show the decreased activity expected when LSK are incubating and the software was unable to distinguish their behaviour prior to and during nesting. This illustrates the importance of variation in individual behaviour with regards to the efficacy of software such as Chick Timer™. It can never be a complete replacement for verifying the incubation status of individuals because it is based on the average behavioural pattern for a given species. Both the birds that showed “abnormal” activity levels during incubation went on to hatch a chick. Any “rules” created for this kind of software for a given species are better described as guidelines and each time a tool like this is deployed in a new species (even within other kiwi species), ground-truthing studies such as this one will be important to establish the limitations of the software and to inform adjustments to its algorithms for that species where possible. In any study of hatching success, great care should

be taken around birds that may be nesting, even if equipment such as a Chick Timer™ tag does not indicate incubation.

Hatching events

While the Chick Timer™ software reliably detected hatches in single-egg nests and could register at least one hatch in a two-egg nest, it was unable to detect multiple hatch events in the same nest. LSK are commonly cited as incubating one egg at a time, with rare instances of two egg clutches (Heather & Robertson 2005). Thus, the NiB version of the Chick Timer™ software was thought to be the most suitable for use in LSK as it is designed for detection of one hatching event. However, we have found higher instances of two egg clutches in LSK than was previously reported (Chapter 3). Modification of the software to detect more than one hatching event would be useful for future studies of LSK and other species with multiple eggs per clutch.

Applications and challenges

Chick Timer™ software provides a disturbance minimizing monitoring tool that allows remote data collection. As such, it is also useful for field sites that cannot be accessed for direct observation on a regular basis. Bird species on islands that can be circumnavigated by boat can be monitored without researchers regularly landing on the island and, in larger areas, aerial monitoring technology can be fitted to light aircraft or helicopters to facilitate data collection and remote monitoring of large numbers of individuals (Wilks & Bramley 2010).

The main limitation currently for the broader use of Chick Timer™ software is its dependence on a two-stage radio transmitter, the smallest of which currently available weighs 11g. For birds smaller than LSK (especially those that fly), the tags required for Chick Timer™ software would currently be too heavy. Tag technology is constantly developing, however, and lighter two-stage transmitters may soon be available. For larger species, Chick Timer™ software is highly adaptable in terms of the type and amount of data that can be collected for an individual, making it suitable for use outside of kiwi. It has already been adapted to monitor mating and incubation in another threatened New Zealand endemic bird, the kākāpō (*Strigops*

habroptila) (J. Wilks, pers. comm., November 2013) and could be used in other ratites, larger species of fowl, raptors and seabirds.

The data provided by the Chick Timer™ software is designed to be an approximation to guide researchers in their activities rather than to completely replace direct observation. Statistics such as the start of incubation or the date of hatch, for example, are usually accurate to within a few days rather than the exact date (J. Wilks, pers. comm., November 2013). This could result in failures very early in incubation being missed, especially in species with shorter incubation periods, but this can be compensated for by using measures of hatching success such as the Mayfield method, which account for nest detectability (Mayfield 1975). Chick Timer™ software can save researchers and managers time, allowing a larger number of individuals to be monitored for hatching success. The resultant capacity to increase sample size and statistical power, reduce disturbance and conduct studies in remote or relatively inaccessible locations makes Chick Timer™ software an attractive option for researchers and conservation managers. We encourage the trialing of Chick Timer™ software in other bird species to enable collection of accurate hatching success data across diverse taxa.

Appendix 2A – How NiB Chick Timer™ tags record activity

Figure 2A.1: Tags used for the NiB Chick Timer™ program contain a small piece of mercury inside a chamber fitted with a “gate”. As the tag moves, this piece of mercury slides forward (A), passes through the gate (B) and into the other side of the chamber (C). The gate is a sensor that detects the movement of the mercury. The sensor reports how frequently the mercury moves back and forth through the gate in a 10 minute period. If the mercury is moving for 17.5% of a 10 minute period, that 10 minute window is counted as being an active window. NiB Chick Timer™ software keeps a record of the number of active 10 minute windows in a 24 hour period and uses this to calculate a daily activity estimate. This activity time is reported in the tag’s data output (Box 2) and used to establish the time the bird became active the previous night. As kiwi activity levels change during incubation and hatching, the software can use these data to establish the status of the bird and, in the case of incubating birds, whether a hatch event has occurred (Appendix 2B). Thus, accurate detection and recording of activity levels are crucial if the software is to output accurate data.

Appendix 2B – Decoding an NiB Chick Timer™ tag output

The NiB Chick Timer™ software output can be divided into two main sources of data, most of which are determined by the bird's activity. The continuous background pulse rate indicates the status of the bird. 30 pulses per minute (ppm) indicate non-incubation, 40ppm indicate incubation and 80ppm indicate the tag has not moved for 24 hours (therefore the bird is dead or has dropped the tag). This background pulse rate is broken every 10 minutes by the data output, a series of faster, coded pulses, which gives more detailed information on the bird's activity in a total of eight outputs (Fig. 2B.1). Thus, researchers can listen to the tag at almost any point and discover the status of the bird being tracked and then wait for the data pulse to gain extra information. The background pulse rates and data outputs can be modified for different species and to accommodate different battery lives. The examples given here are specifically for the LSK-modified NiB Chick Timer™ program.

Figure 2B.1: An example data read out from LSK-modified NiB Chick Timer™ software with an explanation of each of the eight data outputs. Each number represents a series of fast pulses by the tag, with the comma representing a small gap and the forward slash representing five slower “spacer” pulses, which allow the person recording the data to distinguish between outputs. The tag cannot code for a zero (as that would equal silence), so a zero is coded by two pulses. Thus, the researcher must subtract two from each digit in order to decode the tag. Minutes of activity are to be multiplied by 10.

Chapter 3: Population growth is not a suitable measure of translocation success: reduced hatching success suggests cryptic inbreeding depression in a growing translocated population of little spotted kiwi

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Abstract

Translocations are an increasingly prevalent conservation tool, but their success is often hampered by a lack of long-term post-translocation monitoring, which prevents effective management. Specifically, data on vital rates such as reproductive success are necessary to determine the stability and long term viability of translocated populations. New Zealand's little spotted kiwi (LSK) (*Apteryx owenii*) survived a bottleneck of, at most, five birds, but now comprises 1,700 birds distributed across eight translocated populations. The species is thought to be recovering and was recently downgraded to near threatened by the IUCN, but long-term monitoring of LSK populations has been limited to population growth assessment. We conducted the first study of hatching success in translocated LSK populations in two locations, Zealandia Sanctuary and Long Island. Hatching success was significantly lower on Long Island than in Zealandia despite significantly higher reproductive effort among Long Island LSK. LSK were found to be capable of producing more eggs per season than previously recorded, which may explain their rapid demographic recovery to date. Low hatching success on Long Island, however, is suggestive of inbreeding depression resulting from an extreme secondary bottleneck ($N=2$) at translocation and subsequent inbreeding. This issue has been masked by steady population growth resulting from high reproductive success of the founding pair. Our study underscores the importance of long-term monitoring of vital rates post-translocation to discover cryptic challenges for long-term population persistence, such as inbreeding depression, that are not always apparent from population growth rates.

Introduction

Translocation (the intentional movement of individuals from one location to another) to overcome barriers to dispersal has become an increasingly prevalent tool for conservation (Seddon 2010). Causes of translocation failure are often unknown due to a lack of post-introduction monitoring (Fischer & Lindenmayer 2000). This precludes mitigative management of translocated populations and impedes improvement of translocation practices, prompting repeated calls for comprehensive post-translocation monitoring (Hein 1997; Ewen & Armstrong 2007; IUCN/SSC 2013). When post-translocation monitoring does occur, it often takes the form of measuring population growth rate, as this is considered a “catch all” for several vital rates (Nichols & Armstrong 2012). However, it is recommended that specific parameters such as reproduction and survival are also monitored following translocation (Sutherland et al. 2010; IUCN/SSC 2013), because they are key factors in modelling long term population viability (Armstrong et al. 2002).

Two major factors known to influence reproductive success in translocated populations are food availability and inbreeding depression. Translocation sites are normally chosen to be favourable for the species being moved, with predator-free conditions, but limited dispersal opportunities, thus translocated populations may grow to outstrip their food supply (Mackintosh & Briskie 2005). In birds in particular, lack of food has been linked to smaller clutch sizes (Clifford & Anderson 2001; Castro et al. 2003), decreased nest attentiveness by incubators (Martin 1987; Eikenaar et al. 2003), and decreased fledging success (Robb et al. 2008; Wellicome et al. 2013), all of which could hamper the long term success of a translocation. Additionally, translocated populations founded with few individuals are more likely to be inbred due to the effects of small population size (Crow & Kimura 1970). This can lead to inbreeding depression – reduced fitness in the offspring of related compared to unrelated individuals (Allendorf et al. 2013) – which is known to increase population extinction risk (Frankham 1995; Keller & Waller 2002; O'Grady et al. 2006). Measuring population growth alone might overlook issues such as these until such time as the population goes into serious decline, leaving less time for attempts to rectify the problems via management.

Here, we examine hatching success in two translocated populations of little spotted kiwi (*Apteryx owenii*) (LSK) in New Zealand that have previously only been monitored via population growth. LSK survived extinction solely due to a translocation of five birds to Kapiti Island in the early 1900s (Ramstad et al. 2013). The mainland population of LSK was extirpated by introduced predators by the 1980s, while the Kapiti Island population flourished, growing to ~1,200 individuals by 2005 (Heather & Robertson 2005). Since 1982, seven new populations have been established via translocation of Kapiti Island individuals to six predator free islands and one predator-free mainland sanctuary (Colbourne & Robertson 1997, R. Colbourne, unpublished). The total current population is estimated to number ~1,700 birds (H. Robertson, unpublished).

The one previous study on reproductive success in LSK, carried out on Kapiti Island in the 1980s, found that LSK pairs normally produce one egg per year (very occasionally two), with an annual hatch rate of 0.11 chicks per pair (Jolly 1989). With the exception of this study, monitoring of this species has been exclusively via estimation of annual population growth rates, which range from 4-12% (mean = 8.8%)(Colbourne & Robertson 1997; Robertson & Colbourne 2008; Ramstad et al. 2013). As a result of these seemingly successful translocations, the species was downgraded from 'Vulnerable' to 'Near Threatened' by the IUCN in 2008 (Birdlife International 2013), despite remaining conservation reliant and exhibiting extremely low genetic diversity (Ramstad et al. 2010; Miller et al. 2011; Ramstad et al. 2013) Few data are available on LSK vital rates, making long term viability uncertain.

Some translocated populations of LSK are at risk of inbreeding depression due to the low numbers of individuals used to found them. Here, we consider the two populations with the highest and lowest number of founders, Zealandia Sanctuary (40 founders translocated in 2000-2001, currently ~120 birds) (H. Robertson, unpublished) and Long Island (2 founders translocated in 1982 and 1989, currently ~50 birds) (Ramstad et al. 2013, H. Robertson, unpublished). Both populations have shown positive post-translocation growth rates (Zealandia 9.5% and Long Island

11% per year), but Long Island has the lowest genetic diversity of any LSK population studied to date (Ramstad et al. 2013). In this study, we ask:

- 1) What is the level of reproductive effort and success in recently translocated LSK populations and does it differ significantly between sites?
- 2) Do differences in environmental or behavioural variables explain patterns of reproductive success in LSK?
- 3) Is there any indication of inbreeding depression affecting reproduction in the Long Island population?

Materials and methods

Study species

The LSK is a flightless, insectivorous ratite endemic to New Zealand. LSK are nocturnal and spend the daylight hours sheltering in burrows and tree cavities. They are long-lived (life expectancy in the wild of 27-83 years) (Robertson & Colbourne 2004) and socially monogamous, but the male is solely responsible for incubating eggs. LSK nests are also typically in burrows, but with the entrance camouflaged with leaf litter. During incubation, the male's nightly activity is usually reduced until his egg hatches a precocial chick, which remains in the nest with the male for several weeks after hatching (Heather & Robertson 2005).

Study sites

Zealandia is a 225ha mainland island sanctuary in Wellington, New Zealand, consisting of native podocarp and introduced pine forest. The sanctuary is surrounded by a 2.2m high fence that is impervious to the majority of introduced mammalian predators. Long Island is a 160ha ridge of land at the northern end of the Queen Charlotte Sound in Marlborough, New Zealand. The island was cleared of mammalian predators in 1992 although an avian kiwi nest predator, the weka (*Gallirallus australis*), is occasionally present on the island.

Monitoring LSK reproduction and behaviour

We used hatching success as our measurement of reproductive success because reliably determining fledging success and juvenile survival across all nests in both

sites was not feasible. Male LSK were monitored over two consecutive seasons: from June 2011-February 2012 and June 2012-February 2013. This monitoring period encompassed the entire LSK nesting season (September to January, during the austral spring/summer) (Heather & Robertson 2005) while allowing time for birds to be caught and tagged well before nesting began.

At both sites, birds were initially located using trained kiwi tracking dogs. All kiwi capture, handling and tagging was conducted according to the New Zealand Department of Conservation's Kiwi Best Practice Manual (Robertson & Colbourne 2003). Males were fitted with V2L 152A VHF leg band radio tags (Sirtrack, Havelock North, NZ), preloaded with NiB Chick Timer™ V3.5 for little spotted kiwi software (Wildtech Ltd, Havelock North, NZ). Chick Timer™ software is an accurate and effective tool for monitoring LSK activity and hatching success (Taylor et al. 2014) and was used to collect data on the activity, and incubation and hatching status of tagged males. A total of 26 Zealandia birds and nine Long Island birds were monitored (~43% and 36% of each population respectively, accounting for birds being paired) (Table 3.1). Fourteen Zealandia birds and all nine Long Island birds were monitored throughout both seasons to measure inter-annual variation in reproductive effort and success (Appendix 3A – Table 3A.1).

Males were tracked to their burrows during daylight hours using a Telonics TR4 receiver (Telonics, Mesa, Arizona, USA) and Yagi folding antenna (Kiwitrack, Havelock North, NZ). Birds were tracked weekly in Zealandia and every six weeks on Long Island to locate their burrows and record activity and incubation status data from the Chick Timer™ software. Chick Timer™ data from Long Island birds were also collected remotely each month from a boat. Incubation attempts were initially indicated by the Chick Timer™ output and repeated use of a camouflaged burrow by a male. After several weeks of incubation, presence of an egg in a nest was verified with a burrowscope (Taupe infra-red burrowscope, Sextant Technologies, Wellington, NZ) while the male was away from the nest at night. Hatching events indicated by the Chick Timer™ software were verified by direct observation of the chick leaving the nest at dusk.

If males abandoned their nests, the eggs were removed and autopsied to determine the likely cause of egg failure. It was impossible to detect early stage development in abandoned eggs due to the rapid breakdown of tissues. Thus, all eggs lacking signs of development were grouped as infertile/early stage embryonic death (ESD), while eggs containing embryos were classed as either mid or late stage embryonic death (MSD and LSD), depending on the size of the embryo.

Climate

Daily rainfall and minimum temperature from the nearest weather stations for both sites (1 March-28 February for the relevant years) were obtained from CliFo: the National Institute of Water and Atmospheric Research's National Climate Database (<http://clifo.niwa.co.nz>, accessed 19th September 2013). For Zealandia this was the Kelburn weather station (41.285°S 174.768°E), ~1.6km north east of the site and for Long Island, the Pelorous Sound Crail Bay weather station (41.103°S, 173.964°E), ~20km west of the island.

Statistical analyses

Clutch size

Average clutch size (number of eggs per nest per incubating male, including second clutches) was calculated for each breeding season and site. The ratio of one to two egg nests (including second clutches) was compared between seasons and sites using chi-squared tests. For comparison with the previous study on Kapiti Island (Jolly 1989), we calculated ratios of one to two egg nests across seasons for each site, including second clutches.

Hatching success

Hatching success was calculated both as apparent hatching success (AHS) and using the Mayfield estimator to account for any early stage incubation attempts that may have been missed in our monitoring (Mayfield 1961; Mayfield 1975; Johnson 1979). Hatching success analyses were based on first clutches only because Chick Timer™ software is designed to deal with one clutch per season and provided unreliable hatching data for second clutches. Within-population AHS was calculated as the proportion of successful hatches for the total number of first clutch eggs found in

each site in each season. Confidence intervals (95%) for AHS were calculated using the Wilson score method without continuity correction (Newcombe 1998). AHS between sites and seasons was compared using chi-squared tests. For comparison with Jolly (1989), we also calculated the number of hatch events per pair per year pooled across both nesting seasons based on data from first clutches only. Consistency of hatching performance across males between seasons was assessed by comparison to a random binomial distribution with a G likelihood ratio test.

Interactions between environmental and behavioural variables and hatching success

To examine the effect of environmental and behavioural variables on hatching success, we used logistic regression to compare the success of individual nests as a binomial response variable (i.e., whether a nest produced at least one hatched egg or failed). Because some birds were tracked in both seasons, we tested the necessity of a random intercept term for individual bird using likelihood ratio testing in the R packages lme4 (Bates et al. 2013) and nlme (Pinheiro et al. 2012). Addition of this random intercept term did not improve the performance of any of the logistic regression models, so it was removed and the results from the baseline model reported. An additive step-wise multilevel linear model analysis including a random effect for bird was conducted to investigate the relationship between site, activity during incubation and ambient temperature during incubation. Detailed model summaries are presented in Appendix 3A (Tables 3A.2-3A.4). All statistical analyses were carried out in R (R Development Core Team 2013).

Results

Reproductive effort

Nearly all (89% in both seasons) males monitored on Long Island attempted to nest at least once in both nesting seasons compared to 63% in 2011/12 and 81% in 2012/13 in Zealandia (Table 3.1). Clutches consisted of either one or two eggs. There was no significant difference in the incidence of one and two egg nests between seasons within sites (Zealandia $\chi^2_1=2.80$, $P=0.09$; Long $\chi^2_1=0.19$, $P=0.66$), but there was a significantly higher incidence of two egg nests on Long Island than in Zealandia in both seasons (2011/12: $\chi^2_1=9.26$, $P<0.01$; 2012/13: $\chi^2_1=33.80$, $P<0.001$) resulting in a greater mean clutch size on Long Island in both seasons

(Table 3.1). Both sites showed a higher proportion of two egg nests than reported on Kapiti Island (Table 3.2). Hatching success did not differ between one and two egg nests ($\chi^2_1=0.53$, $P=0.47$). There was a trend towards lower nightly activity (and thus more time spent incubating) in two egg nests, but it was not significant within sites or across sites (Welch two-sample t-test: $t_{29}=1.92$, $P=0.06$). “Double clutching” (one male having two separate, consecutive nests in the same season) was observed on Long Island, but not in Zealandia (Table 3.1) and males that incubated second clutches included those with successful and unsuccessful first nests (Appendix 3A – Table 3A.1).

The number of eggs incubated by individual males each season was fairly consistent. For males that were tracked and attempted incubation in both seasons, 56% in Zealandia and 88% on Long Island had the same clutch size in their first nest in each season. Three birds on Long Island had different clutch sizes between first and second nests in the same season. Two Zealandia males that nested in the 2011/12 season were not observed to nest in 2012/13 and one Zealandia male that did not nest in 2011/12 nested in 2012/13 (Appendix 3A – Table 3A.1).

Table 3.1. Reproductive effort and first clutch hatching success for male LSK in Zealandia and on Long Island over two nesting seasons.

Site	Season	# birds monitored	# birds nested at least once	# birds known to have two clutches	Total # first clutch eggs	Apparent hatching success ($\pm$ 95% CIs)	Mayfield hatching success estimate ($\pm$ 95% CIs)	Mean clutch size of incubating birds (all nests)($\pm$ 95% CIs)
Zealandia	2011/12	16	13 (81%)	0	18	78% ($\pm$ 13%)	71% ($\pm$ 23%)	1.4 ($\pm$ 0.3)
	2012/13	24	15 (63%)	0	19	58% ($\pm$ 19%)	60% ($\pm$ 19%)	1.2 ($\pm$ 0.2)
Long Island	2011/12	9	8 (89%)	4	14	43% ($\pm$ 25%)	58% ($\pm$ 32%)	1.8 ($\pm$ 0.1)
	2012/13	9	8 (89%)	2	15	27% ($\pm$ 25%)	27% ($\pm$ 24%)	1.8 ($\pm$ 0.3)

Table 3.2. Comparison of Zealandia, Long Island and Kapiti Island LSK (Jolly 1989). Data on number of eggs per pair per year were not available for Kapiti Island.

Site	# pairs monitored across study	Study period (seasons)	Eggs per pair per year (first clutch only)	Hatches per pair per year (first clutch only)	Ratio of 1:2 egg nests (all clutches)
Zealandia	40	2	0.93	0.63	2 : 1
Long Island	18	2	1.61	0.56	1 : 3
Kapiti Island	12	5	-	0.11	7: 1

Hatching success

Hatching success was higher in Zealandia than on Long Island across both study seasons (Table 3.1). Mayfield estimates of hatching success were similar to AHS (Table 3.1), and so the latter was used for statistical testing. AHS did not differ between seasons within sites (Zealandia $\chi^2_1=2.90$, $P=0.89$; Long $\chi^2_1=1.90$, $P=0.17$), but was significantly higher in Zealandia in both seasons (2011/12: $\chi^2_1=4.90$, $P<0.01$; 2012/13: $\chi^2_1=9.50$, $P<0.01$). The mean number of chicks hatched per pair per year was similar between sites over the two seasons combined, but much higher than reported for Kapiti Island (Table 3.2).

Incubation duration for hatched eggs ranged from 62 to 91 days (mean=77, sd=7) and did not differ significantly between sites or monitoring seasons (Kruskal-Wallis: $\chi^2_1=3.50$, $P=0.32$). Incubation duration for eggs that were abandoned was highly variable. Abandonment occurred from 14 to 63 incubation days for infertile/ESD eggs, at 68 incubation days for the one MSD egg and 83 incubation days for the two LSD eggs.

Some males consistently hatched eggs while others (particularly on Long Island) consistently had eggs that failed. For males that were tracked over and nested in both seasons, 81% in Zealandia and 63% on Long Island showed the same patterns of hatching success or failure each season (Appendix 3A – Table 3A.1). This is significantly different from random in Zealandia ($G_1=4.82$, $P<0.05$), but not Long Island ($G_1=0.50$, $P=0.48$). However, 100% of males in both sites that nested in both seasons were consistent in producing at least one fertile egg containing a detectable embryo (whether hatched or failed) per season or all infertile/ESD eggs (Appendix 3A – Table 3A.1).

Climate

When averaged over the entire year, minimum daily temperature and daily rainfall did not differ significantly between sites in either year (Mann Whitney U: 2011/12 min. temp. $W=59,667$, $P=0.16$; 2012/13 min. temp. $W=43,439$, $P=0.32$; 2011/12 rain $W=66,557$, $P=0.33$; 2012/13 rain $W=49,388$, $P=0.17$). In both seasons, however, minimum daily temperature was significantly lower on Long Island than in

Zealandia during each site's nesting period (first nests only) (Welch two-sample t-test: 2011/12 $t_{245}=8.16$, $P<0.001$; 2012/13 $t_{312}=6.03$, $P<0.001$). There was no significant difference in the average daily rainfall between sites in either season during nesting (first nests only) (Mann Whitney U: 2011/12 $W=10,259$, $P=0.13$; 2012/13 $W=8,355$, $P=0.56$).

Timing of nesting

Male LSK on Long Island commenced incubation earlier than those in Zealandia in both seasons (Fig. 3.1). In 2011/12, all birds on Long Island commenced incubating a first clutch by the end of August, whereas first nests were initiated in Zealandia between September and November. In 2012/13, nearly all first nests were found on Long Island from July through to September (except for one in November), while the timing of nesting was similar in Zealandia to the previous season (Fig. 3.1).

Commencement date of incubation was not related to whether a nest produced a successful hatch (Logistic regression: $\beta=0.01$, $z=1.27$, $P=0.20$) (Appendix 3A – Table 3A.2) and the nesting season was longer on Long Island (205-212 days) than in Zealandia (164-183 days).

Incubation activity

Nightly activity of incubating males ranged from 110 to 510 minutes (mean=303, $sd=70$) and, as expected, was approximately half that of non-incubating males (340-930 minutes, mean=636, $sd=92$) (Fig. 3.2). Mean nightly activity of incubating birds did not differ between seasons at either site (Mann Whitney U: Zealandia $W = 9188$, $P=0.08$; Long Island $W = 1151$, $P=0.30$), but was significantly lower on Long Island by an average of 90 and 60 minutes nightly in 2011/12 and 2012/13 respectively (Mann Whitney U: 2011/12 $W=6854$, $P<0.001$; 2012/13 $W=4214$, $P<0.001$) (Fig. 3.2). The best model for nightly activity included both site and mean minimum ambient temperature, but only site was a significant predictor (Site $\beta=51.78$, $t_{22}=16.30$, $P<0.01$; Ambient temp. $\beta=8.40$, $t_{15}=4.57$, $P=0.09$) (Table 3A.3). The random effect of individual significantly improved the model ($\Delta AICc=-8.06$, $L_1=10.05$, $P<0.01$) and the intraclass correlation coefficient for average nightly activity during incubation within individuals was 0.31. The relationship between

activity during incubation and whether a nest produced a successful hatch was not significant ($\beta=0.00$, $z=-0.41$, $P=0.68$) (Appendix 3A – Table 3A.4).

Causes of egg failure

In both sites, the majority of eggs that failed to hatch were found to be infertile or to have experienced early stage death (Table 3A.5). All embryos that experienced mid and late stage death were malformed (unformed umbilicus), malpositioned, or both. We encountered only one incidence of apparent weka predation or scavenging of a nest on Long Island, where a first clutch egg in 2012/13 containing an embryo had been torn open in a fashion inconsistent with a hatch attempt (E. Bean, pers. comm., November 2012).

Figure 3.1. Timing of incubation onset for first nests of male LSK monitored in Zealandia and on Long Island over two nesting seasons.

Figure 3.2. Minutes of activity per night for incubating and non-incubating male LSK in Zealandia (Z, yellow) and on Long Island (L, blue) over two monitoring seasons. Boxes represent the upper and lower quartiles, divided by the median. The 10 and 90 percent quartiles are depicted by lines and dots represent the outliers.

Discussion

This study demonstrates a clear disparity between reproductive investment and hatching success in the translocated Long Island LSK population, where significantly lower hatching success was observed relative to the Zealandia population despite significantly higher reproductive investment. Clutch size, ambient temperature during incubation, timing of nesting and time spent incubating at night were not significant predictors of hatching success, but site was, suggesting a difference between the two locations affecting hatching success that was not accounted for in our measurements. The significant effort invested in reproduction by LSK on Long Island suggests ample food resources are available in this location. However, the repeated patterns of success and failure for individual males in both sites and the fact that all failed eggs either showed no signs of development or contained a

malformed or malpositioned embryo is highly suggestive of a genetic component to hatching success. Thus, elevated hatching failure on Long Island could be due to inbreeding depression resulting from the extreme bottleneck of two founders used to found this population in the 1980s. The current practice of monitoring LSK populations via population growth rates has failed to detect this problem, illustrating the need for monitoring of specific vital rates in translocated populations.

Our results show that LSK are capable of much higher egg production than previously observed by Jolly (1989), which could partly explain how this species recovered so rapidly from a bottleneck of, at most, five individuals in the early 1900s. The lower incidence of two egg nests in Zealandia and on Kapiti Island may be due either to those populations being at high density, causing increased competition for resources (eg, territory, food or mates) (Both et al. 2000) or resources being more limited in Zealandia than on Long Island in general (Nager et al. 1997). Resource limitation could also explain why a lower proportion of males in Zealandia attempted to nest each year compared to Long Island.

Hatching success in Zealandia and on Long Island was higher than that reported previously on Kapiti Island (Jolly 1989), possibly due to weka predation on Kapiti Island. Data for hatching success in other ratites is scant, but has been reported as 43-56% for brown kiwi (*A.mantelli*), 48% for rowi (*A.rowi*), 62% for tokoeka (*A.australis*) (Robertson et al. 2011b; Robertson & deMonchy 2012), 30% and 60% for greater and lesser rheas respectively (*Rhea Americana*, *R.pennata*) (Navarro & Martella 2002) and 42% for ostriches (*Struthio camelus*) (Sebei et al. 2009). Thus, the hatching rates reported here (27-78%) for LSK are not atypical (although those in Zealandia in 2011/12 are the highest yet recorded for any ratite species in the wild and those for Long Island in 2012/13 the lowest), but the significant difference in hatching success between locations suggests important underlying differences leading to lower hatching success on Long Island despite intense reproductive effort.

The earlier start of nesting on Long Island increased the length of the nesting season, facilitating incubation of multiple clutches, as observed in other avian species

(Verboven et al. 2001). Earlier nesting on Long Island is curious as variables commonly cited as cues for the onset of egg laying in birds such as photoperiod (Dawson et al. 2001) and ambient temperature (Visser et al. 2009) are similar between the two sites. Phenology of the dominant vegetation has also been suggested as a cue for egg-laying (Bourgault et al. 2010), and there are differences in forest composition between the two sites, but this would be an indirect cue for a flightless, ground-dwelling insectivore such as LSK. The most likely explanation for the earlier onset of nesting on Long Island would be food abundance, which is known to advance lay date in great tits (*Parsus major*) (Nager et al. 1997) and which was found to be strongly correlated with lay date for another insectivorous bird, the tree swallow (*Tachycineta bicolor*) (Dunn et al. 2011).

Earlier nesting resulted in lower average minimum ambient temperatures over the incubation periods of birds on Long Island relative to Zealandia. Nesting at lower temperatures requires that incubators spend more time on the nest and less time foraging. In a study of 97 North American passerines, species that nested in colder climates were found to take shorter bouts away from the nest so their eggs did not cool past a critical point (Conway & Martin 2000). LSK on Long Island may be similarly constrained, with significantly lower activity recorded during first incubation for the males in that location. However, ambient temperature was not a significant predictor of activity. The only significant explanatory variable examined for activity was site, suggesting some difference in the sites other than ambient temperature causing lower activity in Long Island birds. Higher food availability on Long Island would allow birds to spend less time foraging each night and more time incubating. This would enable them to nest at colder ambient temperatures and allow nesting earlier in the year, facilitating a longer nesting season and greater egg production via second clutches.

Large investment in reproduction suggests that LSK on Long Island are not limited by food availability or territory space. For birds like kiwi that produce precocial young, egg production and incubation are the most nutrient-limited stages of reproduction (Martin 1987) and increases in food are known to increase egg production in several avian species (Clifford & Anderson 2001; Castro et al. 2003).

Kiwi have one of the longest known incubation periods in birds (Calder et al. 1978); male LSK lose a substantial amount of body weight during incubation and are generally found to be in poor condition at the end of nesting (H. Taylor, pers. obs.). Female kiwi also invest heavily in reproduction, producing one of the largest and most energy-rich eggs in relation to body mass of any bird (Calder et al. 1978). Long Island LSK females can seemingly produce 1-4 eggs per season and males incubate up to two clutches per season (a total of up to 180 days incubation) and spend more time on the nest each night than males in Zealandia, suggesting the energetic and nutritional requirements of these birds are being met by their habitat. Invertebrate survey data is not available for Zealandia and only available for Long Island from before rats were eradicated (Moeed & Meads 1987), so it is not currently possible to directly compare food availability between the two study sites. However, there is no significant difference in the weight of LSK between Long Island and Zealandia (H. Robertson, unpublished) and there was no significant difference in rainfall between sites during this study that might have affected food availability (Colbourne & Kleinpaste 1983).

There was a prevalence of either infertile or early stage deaths in abandoned eggs in both sites. Embryos that had died at the mid or late stage were either malformed (unformed umbilicus), malpositioned or both; any of which would prevent successful hatching in the wild. Infertility and malformed embryos are commonly associated with inbreeding depression in birds (Bensch et al. 1994; Jamieson & Ryan 2000; Brekke et al. 2010). Patterns in these occurrences in individual birds were evident, suggesting there is a genetic, rather than purely environmental, component to hatching success.

There are additional variables that we did not measure that could contribute to egg failure (e.g. age of parental birds, parasite load, microbial content of soil, nest characteristics). However, our data and the genetic history of Long Island LSK are consistent with inbreeding depression in this population. This assertion may seem incongruous with the positive growth rate of the Long Island population to date. However, LSK are a long lived species. The founding male and female on Long Island are known to be at least 33 and 27 years old, respectively, and this pair has hatched

at least three chicks over the two years of this study. When the Long Island founders are removed from our data, AHS at this site drops from 58%(±32%) to 33%(±28%) for 2011/12 and from 27%(±24%) to 15%(±27%) in 2012/13. If hatching failure (or offspring survival) in this species is linked to inbreeding depression, relatively high productivity of the two founders could mask reproduction and survival issues resulting from inbreeding in subsequent generations, making the effects of inbreeding depression particularly insidious. The population could persist and have a positive population growth rate for decades until the founders eventually stop reproducing. At this point, the reproductive problems among closely related breeding pairs and survival and reproductive issues in subsequent inbred generations would be exposed, potentially reducing population growth and causing unexpected failure of a seemingly successful translocation. A similar scenario exists in the extremely long-lived dawn redwood (*Metasequoia glyptostroboides*), where over six hundred million individuals have been bred from founders rediscovered in the 1940s. Genetic analysis suggests only a few founders were used for this process and germination problems in the highly inbred seeds of closely related first generation trees mean that the population could eventually go into decline once the founders have all died (Li et al. 2012).

These results highlight the importance of long-term monitoring of specific vital rates following a translocation. While coarse metrics such as population growth are useful to assess short-term establishment success and more economical to implement than the kind of detailed study carried out here, they have the potential to mask factors that can affect long term persistence. The disparity between reproductive effort and success in the Long Island population is suggestive of inbreeding depression that could affect the long term viability of this population. A better understanding of the genetics of LSK is a priority (Holzapfel et al. 2008) and studies are underway to assess if inbreeding is related to hatching success in both the Long Island and Zealandia populations. We recommend long-term monitoring of vital rates for LSK and translocated populations of other species where possible, especially those with long life-spans where problems in survival and reproduction caused by inbreeding will likely manifest in the distant future rather than the short term. In such a scenario, long generation interval may allow time for management

to mitigate the effects of inbreeding depression, a potentially damaging agent of long-term translocation failure.

Appendix 3A

Table 3A.1: Hatching success and failure and number of confirmed eggs per nest across two consecutive nesting seasons for male LSK monitored in both seasons in Zealandia and on Long Island. H = at least one successful hatch; F = failed to hatch; (E) = embryo present in failed egg.

	Bird	2011/12 Nest 1		2011/12 Nest 2†		2012/13 Nest 1		2012/13 Nest 2†	
		Hatch/ Fail	No. eggs	Hatch/ Fail	No. eggs	Hatch/ Fail	No. eggs	Hatch/ Fail	No. eggs
Zealandia	Z1	F	1	NA	NA	NA	NA	NA	NA
	Z2	F	1	NA	NA	NA	NA	NA	NA
	Z3	F	1	NA	NA	F	2	NA	NA
	Z4	H	1*	NA	NA	H	2	NA	NA
	Z5	H	2	NA	NA	F (E)	1**	NA	NA
	Z6	H	2	NA	NA	H	1	NA	NA
	Z7	H	2	NA	NA	H	1*	NA	NA
	Z8	H	2	NA	NA	H	1	NA	NA
	Z9	H	1	NA	NA	H	1	NA	NA
	Z10	H	1	NA	NA	H	1	NA	NA
	Z11	H	1	NA	NA	H	1	NA	NA
	Z12	H	1	NA	NA	F (E)	1	NA	NA
	Z13	H	2	NA	NA	H	2	NA	NA
	Z14	NA	NA	NA	NA	F	1	NA	NA
Long	L1	H	2	H	1	H	2	NA	NA
	L2	F (E)	2	H	2	H	2	NA	NA
	L3	F	2	F	2	F	2	NA	NA
	L4	H	1	F	2	F (E)	1	NA	NA
	L5	F	2	NA	NA	F	2	NA	NA
	L6	H	2	NA	NA	H	2	NA	NA
	L7	H	1	NA	NA	F (E)	2	F	1
	L8	F (E)	2	NA	NA	F (E)	2	F (E)	2
	L9	NA	NA	NA	NA	NA	NA	NA	NA

†Data from second nests is as complete as possible, but as Chick Timer™ software is only designed to detect one incubation and hatch attempt per season, this data is largely based on direct observation.

*Males suspected to have two eggs due to time spent in nest following hatch, but this could not be confirmed. **Male whose mate was found dead shortly after commencement of incubation of first egg that season (thus potentially precluding a second egg being laid in that clutch).

Table 3A.2: Full results from logistic regression of timing of incubation vs whether or not a nest produced at least one successful egg.

Model	β (SE)	z	P	95% CIs for odds ratio		
				Lower	Odds ratio	Upper
Constant	-0.35 (0.70)	-	-	-	-	-
Incubation start date	0.01 (0.01)	1.27	0.20	0.99	1.01	1.03

$R^2 = 0.03$ (Hosmer-Lemeshow). Model $\chi^2_1 = 1.694$, $P > 0.05$.

Table 3A.3(i) and (ii): Results from multilevel linear model analysis explaining variation in average nightly activity as a function of site and ambient temperature and the interaction of ambient temperature and site as fixed effects. A random intercept term for individual was included in all models except the baseline. (i) Summary of best model. (ii) Model comparison using AIC corrected for small sample size. (Significance codes: *** ≤ 0.001 ; ** ≤ 0.01 ; * ≤ 0.05 ; NS=not significant)

(i)

Fixed effect	β (SE)	t	d.f.	P
Intercept	181.15 (37.65)	4.81	22	***
Site	51.78 (16.30)	3.17	22	**
Ambient temp.	8.40 (4.57)	1.84	15	NS

Random effect = Individual bird: SD =17.98, Residual variance = 27.89

(ii)

Model	AICc	Δ AICc	k	ω
Site+Ambient temp.	403.99	-	2	0.49
Site	404.86	0.87	1	0.32
Site+Ambient temp+Site:Ambient temp.	406.08	2.09	3	0.17
Ambient temp	410.85	6.86	1	0.02
Baseline	429.28	25.29	0	0.00

Table 3A.4: Full results from logistic regression of average nightly activity of a male during incubation vs whether or not a nest produced at least one successful egg.

Model	β (SE)	z	P	95% CIs for odds ratio		
				Lower	Odds ratio	Upper
Constant	1.68 (2.05)	-	-	-	-	-
Average nightly activity	-0.003 (0.007)	-0.41	0.68	0.98	1.00	1.01

$R^2 = 0.004$ (Hosmer-Lemeshow). Model $\chi^2_1 = 0.168$, $P > 0.05$.

Table 3A.5: Stages of mortality for abandoned LSK eggs in Zealandia and on Long Island over two nesting seasons. Mal-positioned embryos are those not in the correct position to facilitate hatching. An unformed umbilicus refers to an unusually wide join between the yolk sac and gut cavity. The two conditions can co-occur. ESD = early stage embryonic death, MSD = mid-stage embryonic death, LSD = late stage embryonic death, MHD = mid-hatch death.

Site	Season	# failed eggs collected	# eggs Infertile/ ESD	# eggs MSD	# eggs LSD	# eggs MHD	# eggs too damaged to autopsy	Within MSD and LSD	
								Mal-positioned	Unformed umbilicus
Zealandia	2011/12	3	2 (67%)	0	0	0	1 (33%)	NA	NA
	2012/13	8	5 (63%)	1 (13%)	0	2 (25%)	0	1	0
	Total	11	7 (64%)	1 (9%)	0	2 (18%)	1 (9%)	1	0
Long	2011/12	13	8 (62%)	1 (8%)	2 (15%)	0	2 (15%)	3	2
	2012/13	13	6 (46%)	1 (8%)	0	3 (23%)	3 (23%)	1	1
	Total	26	14 (54%)	2 (8%)	2 (8%)	3 (12%)	5 (19%)	4	3

Chapter 4: Valid estimates of individual inbreeding coefficients from marker-based pedigrees are not feasible in wild populations with low allelic diversity

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Abstract

Pedigrees are frequently recommended for estimating inbreeding coefficients (F_{PED}), but are error-prone due to missing behavioural data in wild populations. Genetic marker-based pedigrees have been suggested as a remedy to this problem, but their accuracy depends on the number and polymorphism of loci available, and the completeness of population sampling. We used simulations to examine how accuracy of marker-based pedigrees varies with number of loci and sampling regime when allelic diversity is low (2.2-4 alleles per locus in founders), as is often the case in threatened species. We also examined the impact of pedigree errors on the validity of F_{PED} estimated from marker-based pedigrees. Our results indicate that accurate parentage assignments are only feasible if genotypes are available for all individuals that ever existed in the population, and that accuracy does not improve past 40 loci. Errors in marker-based pedigrees resulted in underestimation of F_{PED} by up to 27% and overestimation of the variance in F_{PED} by up to 182%. At least 80% pedigree accuracy was required to produce unbiased estimates of F_{PED} , which were still highly imprecise. Given the degree of sampling required, it is not currently feasible to measure inbreeding in wild populations of threatened species with a pedigree based solely on microsatellite data. Resources may be better directed towards developing more robust genetic tools (whole genome sequencing and large SNP panels) to facilitate direct estimation of inbreeding coefficients without a pedigree. Where this is not possible, long-term monitoring projects will be required to accurately estimate inbreeding coefficients.

Introduction

In normally outbred species, mating between related individuals typically produces offspring that are less fit than those of unrelated individuals (Allendorf & Luikart 2007). This phenomenon, termed inbreeding depression, is a known contributor to population extinction risk (Frankham 1995) and is of particular concern in already threatened species, where small population size leads to increased inbreeding (Crow & Kimura 1970). Accurately measuring inbreeding in species of conservation concern is crucial to estimating their long-term viability (Allendorf & Ryman 2002).

The pedigree inbreeding coefficient (F_{PED}) has been repeatedly recommended as the most effective way to measure inbreeding (Pemberton 2004; Slate et al. 2004; Pemberton 2008; Jones & Wang 2009; Taylor et al. 2010; Grueber et al. 2011). This metric uses pedigree data to estimate the proportion of an individual's genome that is identical by descent (IBD) as a result of shared common ancestry (Wright 1922; Malécot 1948). An F_{PED} of zero represents a completely outbred individual with no part of its genome IBD whereas an F_{PED} of one represents a completely inbred individual whose entire genome is IBD.

Pedigrees can be based on behavioural observations, genetic data, or a combination of the two. The biggest challenge for many researchers seeking to build pedigrees for wild populations is often the lack of behavioural observations to reconstruct the relationships of individuals in the first place (Pemberton 2008; Jones & Wang 2009). Inaccuracies and gaps in a pedigree will lead to invalid estimates of F_{PED} , which could prove highly misleading if applied to detecting inbreeding depression, thus it is important that the pedigree be as accurate as possible (Pemberton 2008). In cases where behavioural observations are lacking, individual genotypes can be used to supplement existing pedigree data (Blouin 2003; Gelatt et al. 2010; Goncalves da Silva et al. 2010) or to build a pedigree de novo (Koch et al. 2008; Wang & Santure 2009; Mourier et al. 2013). Programs that use likelihood-based approaches to infer relationships between individuals based on their genotypes such as CERVUS (Marshall et al. 1998), FAMOZ (Gerber et al. 2003) and COLONY (Jones & Wang 2010) facilitate this process.

Alternative marker-based strategies are available to estimate inbreeding and relatedness directly from genotypes without the need for a pedigree (see Wang 2011 for a summary). However, these tools still require larger numbers of polymorphic markers than are commonly used in conservation genetics studies (Blouin 2003; Santure et al. 2010). Even when sufficient markers are available, estimates depend on the reference population used in the analysis (Wang 2014), and the reliability of these techniques is debatable, especially for populations that have experienced genetic bottlenecks (Robinson et al. 2013). Moreover, pedigrees have uses outside of inbreeding analysis, including estimating heritability of traits (Thomas 2005), analysing mating systems (Engh et al. 2002), assessing the reproductive success of captive-bred versus wild individuals (Read et al. 2012) and detecting extra-pair parentage (Akçay & Roughgarden 2007), the last of which can only be conducted using a molecular approach. Thus, pedigrees remain a popular tool.

Accurate parentage assignments are key to reconstructing a pedigree that will produce valid estimates of inbreeding. Several studies have examined how the accuracy of parentage assignments are affected by variables such as the number and allelic diversity of microsatellite loci, completeness of sampling of individuals in the population, and genotyping error, using both empirical (Estoup et al. 1998; Marshall et al. 1998; Nielsen et al. 2001; Koch et al. 2008) and simulated data (Bernatchez & Duchesne 2000; Harrison et al. 2013). The consensus of these studies is that number and allelic diversity of loci are by far the most important factors in determining the reliability of parentage assignments followed distantly by the completeness of sampling and then genotyping error.

To our knowledge, the effect of number and allelic diversity of loci, completeness of sampling on the validity of F_{PED} estimated from pedigrees reconstructed using genotypes has not been evaluated. Additionally, no studies have specifically focussed on which factors impact most on pedigree reconstruction at the very low allelic diversities characteristic of bottlenecked populations (e.g.: 1.0, 1.9, 2.3 and 3.2 alleles per microsatellite locus in kākāpō (*Strigops habroptilis*), Madagascar fish eagles (*Haliaeetus vociferoides*), Mauritius kestrels (*Falco punctatus*) and South

Island saddlebacks (*Philesturnus c. carunculatus*) respectively) (Jamieson et al. 2006; Johnson et al. 2009), although the effect of low allelic diversity is examined to an extent in Bernatchez and Duchesne, (2000). Finally, it remains unclear whether using more than 20 microsatellite markers (as is now increasingly feasible) could overcome the problem of low allelic diversity. Here, we seek to address these questions using New Zealand's little spotted kiwi (LSK) (*Apteryx owenii*), as a case study.

A flightless ratite, LSK is a species of conservation concern with no behavioural pedigree data whatsoever. All mainland populations of this species were extirpated by introduced mammalian predators by the 1980s. Subsequently, LSK have been managed by founding new populations on seven different island and mainland sanctuaries from one stronghold population on Kapiti Island (Colbourne & Robertson 1997, Colbourne 2012, unpublished data). Quantifying inbreeding, testing for inbreeding depression and identifying birds genetically optimal for future assisted colonizations and transfers between populations is of high importance for the management of LSK, but no pedigree information is available for the source population on Kapiti Island. For the other seven populations, no pedigree data are available beyond the identities of the founders. Genetic diversity in this species is particularly low due to a bottleneck of, at most, five individuals, 100 years ago (Ramstad et al. 2013). Pedigrees for each population would be very useful, but the low allelic diversity in LSK presents a challenge for reconstructing pedigrees for this species using genetic markers.

Our primary goal is to test if it is feasible to use pedigrees reconstructed with microsatellite genotypes to estimate inbreeding coefficients in LSK, as well as other genetically depauperate species. We use a simulation approach based on empirical genotypes and life-history parameters for LSK to ask the following questions:

- 1) How is the accuracy of parent-offspring assignments affected by the number of loci used to construct a pedigree, the number and genetic variation of founders, and the completeness of population sampling when allelic diversity is low?

- 2) How does the validity (bias and precision) of F_{PED} vary with the accuracy of the pedigree used to estimate it?

Methods

The simulation model

Simulations were conducted using a customised version of an individual-based simulation model (Kardos et al. 2013) for the program R version 3.0.1 (R Development Core Team 2013). The model allows user-specification of founder number, mating system, founder genotypes, reproduction and survival rates, age and size of the final population and produces full pedigrees and simulated individual genotypes at user-defined numbers of microsatellite loci. IBD is held constant for any given value of F_{PED} , resulting in zero variation in genome-wide homozygosity among individuals with the same F_{PED} .

All model parameters were based on known aspects of LSK biology. Thus, the mating system selected was monogamy (Heather & Robertson 2005) with random mating and the possibility to re-mate following the death of a partner (H. Taylor, unpublished). Individuals were programmed to reach sexual maturity at three years of age (Ramstad et al. 2013) and populations were closed with no migration. Twenty five replicate simulations were conducted for each population scenario. To measure the effect of the number of microsatellite markers used to reconstruct a pedigree on F_{PED} estimated from it (RF_{PED}), individual genotypes were sampled from each of these 25 replicates with 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 loci, creating a total of 250 datasets per population scenario.

Population scenarios

The effect of number and genetic diversity of founders on the validity of RF_{PED} was investigated using three population scenarios (Table 4.1). The first two scenarios were based on the Long Island LSK population and the third was based on the Zealandia LSK population. All extant Long Island LSK arose from two Kapiti Island birds translocated to Long Island in 1982 and 1989 (Ramstad et al. 2013) and the current estimated population size is 45-50 birds (H. Robertson unpublished). The Zealandia population of LSK was founded by the translocation of 40 LSK from Kapiti

Island in 2000 and 2001 (20 birds in each year) and the estimated population size is currently ~120 individuals (H. Robertson unpublished). Simulation parameters were designed to match the demography of these two populations as closely as possible. Thus, Long Island-based simulations were founded with two individuals (one male and one female) and run for 25 years, with final population sizes of 40-60 birds and Zealandia-based simulations were founded with 34 birds (19 male and 15 female, based on the availability of empirical founder genotypes – see below) and run for 13 years with final population sizes of 110-130 birds.

Hatching success for each population was based on figures recorded for the 2011/12 and 2012/13 nesting seasons in each site (Chapter 3). Survival probabilities for chick, juvenile and adult life stages were based on estimates for kiwi in predator-free environments (Robertson & Colbourne 2004; Robertson et al. 2011b) and kept constant for non-founders. LSK are long-lived (life expectancy in the wild 27-83 years) (Robertson & Colbourne 2004). Both founders on Long Island and the majority of founders in Zealandia are known to still be alive so founding individuals in the simulations were programmed to live to the end of the simulation.

In the two Long Island and Zealandia-based simulations, founders were assigned genotypes based on the actual genotypes of the founders of each real population. Empirical founder genotypes were available for 15 polymorphic microsatellite markers for Long Island founders and 21 polymorphic microsatellite markers for Zealandia founders (Ramstad et al. 2010; Ramstad et al. 2013, Chapter 5). Six founders from the Zealandia population have never been sampled for DNA and so their genotypes were not available for the simulation founders. Thus, the simulated Zealandia populations were founded with 34 rather than 40 birds. The allele frequencies of these empirical genotypes were used to simulate genotypes consisting of 100 loci by assuming the same allele frequencies for each replicate of 21.

Table 4.1. The six population scenarios used to generate “true” pedigrees and genotyped individuals entered into COLONY for pedigree reconstruction based on molecular markers. *Emp=empirical, Sim=simulated. F_{PED} =Pedigree inbreeding coefficient.

Scenario	Population based on	# founders	Final population size	Founder genotypes*	Mean alleles per locus in founders	Sampling regime	Average proportion birds sampled of those that ever existed (95% CIs)	Average F_{PED} in final population (95% CIs)
A	Long Island	2	40-60	Emp	2.2	All birds that ever lived	1.0	0.022 (0.005)
B	Long Island	2	40-60	Sim	4		1.0	0.022 (0.005)
C	Zealandia	34	110-130	Emp	2.5		1.0	0.003 (0.001)
D	Long Island	2	40-60	Emp	2.2	Only living birds	0.58 (0.02)	0.022 (0.007)
E	Long Island	2	40-60	Sim	4		0.57 (0.02)	0.022 (0.007)
F	Zealandia	34	110-130	Emp	2.5		0.63 (0.02)	0.003 (0.001)

Allelic diversity affects the accuracy of parental assignments in pedigree reconstruction (Bernatchez & Duchesne 2000). As allelic diversity in the real Long Island and Zealandia founders is known to be especially low (mean alleles per polymorphic microsatellite locus = 2.2 and 2.5 respectively), a third scenario (B and E, Table 4.1) was developed with simulated genotypes for the Long Island founders that generated the maximum amount of genetic diversity possible with two individuals (i.e., each of the two founders being heterozygous for unique alleles at each locus). All other parameters in this scenario were identical to the other scenarios (A, C, D and F, Table 4.1).

Sampling regimes

To examine the effect of genetic sampling protocols on the validity of RF_{PED} , individual genotypes simulated in the three scenarios described above were sampled using two different regimes (Table 4.1). The first involved sampling genotype data from all birds that had ever lived in the population (representing complete genetic monitoring since the founding of the population). The second involved sampling only the individuals left alive at the end of the simulation (representing a discrete, comprehensive sampling effort in the present day). The combination of two sampling regimes with three population scenarios produced a total of six models with 250 datasets each for use in reconstructing pedigrees using molecular data (Table 4.1).

Pedigree reconstructions

We selected the program COLONY to reconstruct pedigrees using simulated genotypes because its input parameters are flexible (Jones & Wang 2010) and its full-likelihood approach has been found to outperform two other assignment methods (FAM0Z and exclusion-Bayes theorem) (Harrison et al. 2013). Genotypes were entered into COLONY to produce reconstructed pedigrees for each simulation in each scenario based on different numbers of microsatellite loci (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100). Male and female candidate parent genotypes were entered separately and all genotypes except those of the founders were entered as potential offspring. The parameters for COLONY reconstructions were set as female and male polygamy (necessary to allow for re-mating following the death of a partner), with

inbreeding, in a dioecious and diploid species, medium length of run, full-likelihood analysis at medium precision, and with no sibship prior. Genotyping error was kept constant at 0.01 as this has been found to be a less important factor in assignment accuracy (Harrison et al. 2013) and is likely to be less prevalent when allelic diversity is low (Hoffman & Amos 2005).

COLONY allows the user to specify the probability that the true parent is in the given sample, allowing the software to account for incomplete sampling. COLONY will assign a “novel” parent in cases where the maximum likelihood algorithm determines that the correct parent is not in the sample of candidate parents provided. In reconstructions with incomplete sampling, probabilities that candidate fathers and mothers were present in the sample were input as the proportion of males or females alive of those that had ever lived. Where sampling was complete, these probabilities were set to 1.0 (i.e., a certainty).

Statistical analyses

The structure of the pedigrees reconstructed by COLONY was taken from the best maximum likelihood configuration for each simulation input. These were then compared with the known pedigrees from the corresponding simulations (true pedigrees) by examining the number of correct parent pair-offspring assignments and the number of correct full sibling assignments in each reconstruction. Parent pair assignments were deemed as correct, incorrect or ambiguous. Correct assignments were those where both parents were correctly inferred from those present in the candidate parent sample. In cases where COLONY correctly concluded that the true parent was not in the sample of genotypes provided and assigned a novel individual, the assignment was termed “ambiguous” as there was no way to be certain that the novel inferred individual shared the same heritage as the true parent. When COLONY inferred a novel individual when the real parent was included in the sample, this assignment was deemed incorrect as were assignments where the wrong parent was assigned from within the pool of candidate parents. Ambiguous assignments cannot occur in instances where COLONY is provided with a sample containing all possible parents (i.e., the complete sampling scenarios).

A generalized linear model (GLM) framework was used to quantify the effect of number of loci, sampling regime, number of founders and allelic diversity of founders on the accuracy of parent pair-offspring and full sibling assignments. As the response variable was a proportion (and therefore bounded by 0 and 1), GLMs were fitted using a logit link function and a binomial distribution (Zuur et al. 2009). Models were constructed in an additive, step-wise fashion driven by hypotheses derived from initial data exploration. As number of founders and allelic diversity of founders were co-linear, they were never included in the same model. Details of final models and AIC tables used in model selection can be found in Appendix 4A.

RF_{PED} and the true F_{PED} (TF_{PED}) were calculated using the kinship2 package of the program R (Therneau et al. 2011) and the reconstructed and true pedigrees respectively. The validity of RF_{PED} was measured by calculating the root mean squared error (RMSE) of RF_{PED} compared to TF_{PED} . RMSE accounts for both bias and precision (in the form of variance) of an estimator because $RMSE^2 = bias^2 + variance$ (Wang 2007). Thus, RMSE can be used as a measure of the validity of estimates, but cannot measure how much error stems from bias and how much from imprecision. To account for this, the bias of RF_{PED} was also measured by calculating the difference between RF_{PED} and TF_{PED} for each individual within a scenario at a given number of loci and the variance in RF_{PED} was compared with the variance in TF_{PED} for each scenario to examine the contributions of bias and precision to RMSE. Linear regression was used to investigate the relationship between assignment accuracy and the RMSE of RF_{PED} .

Results

COLONY performance

From the 1,500 reconstructions created using COLONY, 199 (13%) were impossible pedigrees (i.e., an individual was its own ancestor). The majority of impossible pedigrees occurred in instances where lower numbers of loci were used to reconstruct pedigrees, except in scenario D, where the occurrence of impossible pedigrees was relatively even across different numbers of loci. This is a known issue in COLONY when the generation individuals belong to cannot be identified prior to pedigree reconstruction (J. Wang, pers. comm., 27th May 2013). Impossible

pedigrees were not included in analyses of the validity of RF_{PED} , but were included in analyses of the number of correct relationship assignments.

In a total of 28 instances, COLONY failed to complete a reconstruction within 48 hours. The average run time for a reconstruction was approximately two hours and any reconstructions that were not completed within 48 hours were abandoned due to a lack of computational resource to run them for longer. All reconstruction failures occurred in scenario C (34 founders and complete sampling) and the majority (20) occurred for the 10 loci reconstructions.

Accuracy of parentage and full sibling assignments

Overall, parentage assignment accuracy was most strongly influenced by whether genotypes were sampled from all individuals in the population (complete) or just those alive at the end of the simulation (incomplete sampling) (GLM: $\chi^2=170.4$, $P<0.001$). The number of loci used in reconstructions was also an important variable ($\chi^2=91.5$, $P<0.001$) (Fig. 4.1a and table 4A.1a) and, together, these two factors accounted for 50% of the variance in parent pair-offspring assignment accuracy. With complete sampling and 30 or more loci, it was possible to achieve almost 100% accuracy in parent pair-offspring assignments, whereas with incomplete sampling, the maximum achievable accuracy ranged from ~65% (two founder scenarios) to ~85% (50+ loci, 34 founder scenario) (Fig. 4.1a). Allelic diversity of the founders was also a significant predictor of parentage assignment accuracy, but only accounted for 1.8% of the variance ($\chi^2=9.1$, $P<0.01$). As expected, there were no ambiguous parent pair-offspring assignments in scenarios with complete sampling (Appendix 4B). In the scenarios with incomplete sampling, ambiguous assignments were more prevalent in the two-founder scenarios than in the 34 founder scenario (Appendix 4B).

Figure 4.1. a) Proportion of correct parent pair to offspring assignments in reconstructed pedigrees; b) Proportion of correct full sibling assignments in reconstructed pedigrees; c) RMSE of RF_{PED} calculated from reconstructed pedigrees; d) Bias of RF_{PED} (the difference between RF_{PED} and TF_{PED} for each individual) under the six simulation and sampling scenarios. Open points represent scenarios where all individuals were sampled and closed points represent those where only individuals alive at the end of the simulation were sampled. Scenarios presented are the Long Island scenario with two founders and empirical genotypes (■ and □); the Long Island scenario with two founders and simulated genotypes (● and ○); the Zealandia scenario with 34 founders and empirical genotypes (▲ and △). Error bars = 95% confidence intervals. X-axis values offset for clarity.

When between 10 and 30 loci are used, number of loci becomes the most important factor in determining accuracy ($\chi^2=69.4$, $P<0.001$). This is closely followed by sampling regime ($\chi^2=61.4$, $P<0.001$) and, together, these two factors account for 64% of the variance in parentage assignment accuracy in this part of the dataset. Allelic diversity of the founders also plays a more significant role in determining accuracy of parentage assignments when 30 loci or less are used, accounting for 15% of the variance ($\chi^2=29.44$, $P<0.001$). Conversely, once 40 or more loci are used, the number of loci has no effect on accuracy of parentage assignment (Fig. 4.1a) and sampling regime becomes the main explanatory variable, accounting for 54% of the variance in accuracy in parentage assignment on its own ($\chi^2=117.6$, $P<0.001$), with a small effect of the number of founders, which accounts for 5% of the variance ($\chi^2=11.19$, $P<0.001$). Thus, the effect of the other variables on parentage assignment accuracy is highly dependent on the number of loci used for pedigree reconstruction.

The accuracy of full sibling assignments was greater than that of parent-pair assignments, with 100% accuracy achieved for all scenarios when 40 or more loci were used (Fig. 4.1b). The lack of variance between scenarios past this point made it uninformative to build a model for the 40-100 loci subset of data. The models for all loci and 10-30 loci were almost identical (Tables 4A.1d and e) as all the variance in the whole sibling dataset comes from the 10-30 loci subset of the data. Both models suggest that the number of loci used is the most important factor in determining accuracy of full sibling assignment, but this only accounts for 13% of the variance ($\chi^2=45.42$, $P<0.001$).

Validity of RF_{PED}

Close to zero RMSE for RF_{PED} was achievable with complete sampling when the accuracy of parent-offspring assignments was 80-100% (i.e., >30 loci were used in pedigree reconstructions) (Figs. 4.1a and c). The exceptions to this were scenario C with 10 loci, where RSME was not significantly different to that for scenarios A and B despite mean accuracy of parent-offspring assignments being less than 30%, and scenario F, where RSME was as low as for the complete and incomplete sampling scenarios, despite the mean accuracy of the underlying parent-offspring assignments ranging from 12-85% (Figs. 4.1a and c). For scenarios with two

founders and incomplete sampling, parent-offspring assignment accuracy never got above 70% and mean RMSE never dropped below 0.19, even when 100 loci were used to build pedigrees (Figs. 4.1a and c). Linear regressions showed a significant, yet weak ($\beta = -0.03$ to -0.34) negative relationship between correct parent-offspring assignments (i.e., accuracy of reconstructed pedigrees) and RMSE of RF_{PED} for all scenarios (Fig. 4.2).

The negative relationship between pedigree accuracy and RMSE seen in the regressions reflects decreases in bias and/or increases in precision of RF_{PED} as pedigree accuracy improved. The separate analysis of bias in RF_{PED} showed that the complete sampling and 34 founder scenarios (A, B, C and F) all show zero bias, regardless of how many loci are used for pedigree reconstruction (Fig. 4.1d). This means that any RMSE values greater than zero for these scenarios are caused purely by imprecisions in RF_{PED} (i.e., under or overestimating the variance of TF_{PED}). Precision for these four scenarios is generally high, but still variable (Fig. 4.3). The significantly higher RMSE values for the scenarios with two founders and incomplete sampling (D and E) are due to a combination of downwardly biased RF_{PED} values (as low as -0.23 on average for 10 loci in scenario D – Fig. 4.1d) and consistent underestimation of the variance in TF_{PED} (Fig. 4.3). Both bias and imprecision in these two scenarios improved as more markers were used: up to ~ 20 loci in scenario E and ~ 30 loci in scenario D. Past these points, the use of additional markers resulted in little improvement in bias, precision or, consequently, RMSE (Figs. 4.1c, d and 3).

Figure 4.2. Linear regressions of correct parent pair assignments vs RMSE of RF_{PED} for each simulation scenario.

Figure 4.3. Variance in pedigree inbreeding coefficient estimated from true (TF_{PED}) and reconstructed (RF_{PED}) pedigrees under the six simulation and sampling scenarios in Table 4.1. Boxes represent the upper and lower quartiles, divided by the median. Variance in TF_{PED} in each scenario is represented by a shaded box. The 10 and 90 percent quartiles are depicted by lines and dots represent outliers. RF_{PED} is precise when variance in RF_{PED} matches variance in TF_{PED} .

Discussion

Detecting inbreeding depression in species of conservation concern is important and requires error-free measurements of individual inbreeding coefficients. Pedigrees are frequently recommended as the best tool for providing valid estimates of inbreeding, but, when based on behaviour alone, are prone to error due to missing data and extra-pair fertilizations (EPFs). Marker-based pedigrees have been suggested as a remedy to the problems of traditional behavioural pedigrees, but are themselves error-prone when the information content of markers is low, as is true for threatened species with low genetic diversity. We used simulated pedigrees based on real data for little spotted kiwi (LSK), a species with extremely low allelic diversity, to assess the effects of sampling completeness and the number of microsatellite loci available on the accuracy of marker-based pedigrees. We then evaluated the impact of errors in marker-based pedigrees on the validity of inbreeding coefficients estimated from those pedigrees.

Accuracy of parent-offspring and full sibling assignments

Previous studies have suggested that the number of loci available is always the most important factor in parentage assignment accuracy and that a sufficient increase in the number of loci used can overcome problems caused by incomplete sampling or low allelic diversity (Harrison et al. 2013). Our results reiterate the importance of having sufficient loci for pedigree reconstruction, but highlight that when allelic diversity is low (as is often the case for threatened species), completeness of sampling is of much greater importance to pedigree accuracy than previously suggested.

Regardless of the number of loci used, pedigrees reconstructed with incomplete population sampling levelled off at an average of ~67% (2-founder scenarios D and E) and ~80% (34-founder scenario F) parent pair-offspring assignment accuracy. This asymptote occurred at ~40 loci, suggesting that developing more markers than this would be futile. Close to 100% assignment accuracy was only achievable when the entire population that had ever existed was sampled (scenarios A-C), regardless of the number of founders.

Incomplete sampling introduces errors into pedigrees by increasing uncertainty in assignments and introducing ambiguous assignments, which create inaccuracies in pathways through the pedigree. Ambiguous individuals have no ancestors in the pedigree and effectively act as additional founders. Their presence decreases the depth of the pedigree and increases downward bias in RF_{PED} . The improved accuracy in parental assignments in scenario F versus scenarios D and E is likely due to the fact that scenario F ran for 13 years, whereas D and E both ran for 25. As a result, sampling all individuals alive at the end of each 34 founder simulation captures a greater proportion of the total population that ever existed (average 63%) than in the two-founder scenarios (average 57% and 58%). This difference, though seemingly slight, leads to fewer ambiguous assignments (Appendix 4B), and improved accuracy.

Full sibling assignments were consistently more accurate than parent-offspring assignments; close to 100% accuracy in full sibling assignments is achievable as long as 20-30 microsatellite loci are available. Accuracy of ~95% can be obtained with allelic diversity in the founders as low as four alleles per locus and just 10 microsatellite loci for genotyping, even when sampling is incomplete. This is, in part, due to the fact that ambiguous parental assignments have no effect on full sibling assignment accuracy. It does not matter if a “novel” parent is inferred, or even if a parent is incorrect, as long as that parent is consistent across real siblings. This is encouraging for projects wishing to use sibship analysis to monitor dispersal (Lepais et al. 2010), or control breeding stock in a captive or heavily managed population (Austin et al. 2012; Ringler 2012). It is unhelpful, however, for inbreeding studies, where knowledge of ancestry rather than sibling cohorts is required to estimate F_{PED} .

Validity of RF_{PED}

Errors in pedigrees are known to cause error in estimates of F_{PED} , which can, in turn, bias estimates of inbreeding depression and genetic load (Reid et al. 2013). Where we found pedigree accuracy to be lowest (in scenario D with two founders, low allelic diversity and incomplete sampling), mean RMSE of RF_{PED} was between 0.19 (± 0.03 95% CIs) and 0.27 (± 0.02 95% CIs). This represented a downward bias in RF_{PED} of between -0.14 (± 0.01 95% CIs) and -0.23 (± 0.01 95% CIs) and an average

underestimation of the variance in TF_{PED} of between 23% and 93%. While improving the accuracy of the underlying pedigree did reduce RMSE, the magnitude of this reduction was insubstantial, as illustrated by the weak relationship between parent-offspring assignment accuracy and RMSE. For example, in scenario D, increasing the number of loci used in pedigree reconstruction improved the average accuracy of pedigree reconstructions by 50%, but decreased the mean RMSE of RF_{PED} by just 18%.

Even with close to 100% accuracy in the pedigree, RMSE of RF_{PED} was still significantly higher than zero. Although bias was zero when accuracy was high, there was still between 3% (scenario B, 60 loci) and 182% (scenario C, 10 loci) error in estimation of variance in TF_{PED} in scenarios A-C and F, rendering individual RF_{PED} values unreliable. In reality, zero bias would be less achievable due to variance in IBD in individuals with the same TF_{PED} resulting from linkage and recombination during meiosis. This could potentially decrease the accuracy of parent pair-offspring assignments, introducing more bias. Even if pedigrees were 100% accurate, the variance in IBD in individuals with the same TF_{PED} would result in underestimation of variance in F . This was not simulated in our populations as IBD was kept constant across individuals with the same TF_{PED} . An interesting future direction would be to take this “Mendelian noise” into account and measure its effect on the validity of RF_{PED} .

The occurrence of zero bias and very low RMSE in scenario F, which had incomplete sampling and pedigrees that were never more than ~80% accurate, seems curious initially. However, these low RMSE values are likely due to the larger number of founders for scenario F (N=34) vs scenarios D and E (N=2). This resulted, on average, in lower inbreeding coefficients and less variation in TF_{PED} (Table 4.1). RF_{PED} , when biased, consistently underestimated F_{PED} . In a population where the majority of individuals have an F_{PED} of or close to zero, negative bias is not possible and RF_{PED} will be unbiased by coincidence.

Implications

Our results suggest that parent-offspring assignment accuracy of at least ~80% is required to provide estimates of F_{PED} that do not violate the assumption of inbreeding coefficients being measured without error. When allelic diversity is between 2.2 and 4 alleles per locus, this is only achievable with complete sampling or in populations where average F_{PED} is close to zero. In this study, we did not attempt to assess the impact of invalid RF_{PED} on power to detect inbreeding depression. However, in a recent study, Reid et al. found that 28% mis-assignments of paternity in song sparrows (*Melospiza melodia*) due to EPFs in a behavioural pedigree resulted in biases in RF_{PED} that caused underestimation of the effect of inbreeding on annual and lifetime reproductive success as well as juvenile survival by up to 550% (Reid et al. 2013).

Both the complete and incomplete sampling scenarios simulated here are unrealistic for wild populations that are not already part of a long-term monitoring project – even an intensive sampling effort in the present day is unlikely to sample every single individual currently living (although see Ursprung et al. 2011 for an example of "exhaustive" DNA sample collection within a population). In populations with short generation times and high turnover, single-effort genetic sampling, however comprehensive, is likely to produce a very shallow pedigree, which has been shown to produce highly inaccurate estimates of F_{PED} (Balloux et al. 2004). Thus, for the majority of wild populations with low genetic diversity, marker-based pedigrees are likely to feature high degrees of error, which will result in unreliable estimates of F_{PED} .

Further work on how accuracy thresholds for valid RF_{PED} values are affected by the complexity of a pedigree (i.e., its depth and the size of the population) would be informative. The location of errors in a pedigree will also affect the validity of RF_{PED} . Errors deep in the pedigree will cause RF_{PED} to underestimate TF_{PED} for the majority of individuals, but errors later in the pedigree will only create bias in the most recent generations. A useful future step would be to establish whether errors in reconstructed pedigrees occur in random locations within the pedigree and, if not, what factors affect their position. Programs such as PEDANTICS (Morrissey et al. 2007;

Morrissey & Wilson 2010), in conjunction with the simulation-based modelling approach implemented here could facilitate such a study.

Reliable pedigrees can be built when long-term observational and genetic data can be combined (e.g. Nielsen et al. 2012). We did not include behavioural data in our reconstructions and so the effects of this on pedigree accuracy for LSK are unclear. Researchers looking to conduct pedigree reconstruction for a given population should consider the feasibility of intense behavioural and genetic sampling, as well as the number and allelic diversity of genetic markers available to them. We based our simulations on vital rates specific to LSK and would encourage researchers working on different species with different life histories to determine their power to build marker-based pedigrees to estimate inbreeding by running their own simulation-based modelling experiments.

In the case of the two LSK populations modelled here, we have no behavioural pedigree information and genotypes for approximately 78% and 85% of the current populations – less than in any of the scenarios depicted in our experiments. With the 21 polymorphic microsatellite markers currently available, it might be possible to produce estimates of F_{PED} for the Zealandia population (which has 40 founders and likely low average F_{PED}) that were non-biased on average, but there would likely still be high imprecision in RF_{PED} . For the Long Island population, where RF_{PED} is likely to be biased due to high inbreeding and incomplete sampling, the simulation-based modelling conducted here could be used to estimate a correction factor to remove the bias. The precision of RF_{PED} would remain low, however, and thus RMSE would remain high, even when bias was accounted for and corrected. For LSK, and other species with similar allelic diversity, using a microsatellite marker-based pedigree to estimate F_{PED} and assess inbreeding depression would be unwise.

Problems with using pedigrees to estimate the proportion of an individual's genome that is IBD can be divided into two main areas: the inability to construct the pedigree accurately and problems with F_{PED} itself. We are concerned here with the former, but it is important to note that F_{PED} is a potentially unreliable predictor of IBD. First, the calculation of F_{PED} assumes that the founders of the pedigree are unrelated,

which is rarely the case and will lead to underestimation of inbreeding coefficients (Jones et al. 2002; Russello & Amato 2004). Second, F_{PED} does not account for the variance in IBD between individuals with identical F_{PED} that occurs due to chance (Hill & Weir 2011; Forstmeier et al. 2012). Both these problems can be overcome by the use of high density single nucleotide polymorphism (SNP) panels and whole genome sequencing (WGS) to measure inbreeding coefficients directly, without the need for a pedigree (Keller et al. 2011; Purfield et al. 2012, Kardos et al., 2014 in review). However, these tools are not currently as readily available as microsatellite markers for the majority of non-model species that feature in endangered species listings.

Pedigrees have a variety of uses, from estimating heritability to detecting extra-pair parentage, and are thus likely to remain a popular tool. We do not recommend the use of pedigrees to measure inbreeding coefficients in wild populations. The amount of data required to produce accurate pedigrees and valid estimates of inbreeding is not currently feasible to obtain for most studies of wild populations. Direct marker-based estimators of inbreeding and relatedness eliminate the need for a pedigree or complete population sampling and so could prove useful in situations where complete samples are unavailable. However, the validity of estimates produced by these methods is similarly constrained by the number and allelic diversity of the markers available (Wang 2006). A more useful approach would be to direct resources into whole genome sequencing or at least constructing large SNP panels for non-model organisms, the benefits of which would extend far beyond the measurement of inbreeding (Allendorf et al. 2010). Until those data are available, conservation managers interested in detecting inbreeding depression in species with low genetic diversity would perhaps be best served by investing resources in long term behavioural monitoring (combined with comprehensive genetic sampling), which would also reap multiple benefits for conservation (Clutton-Brock & Sheldon 2010).

Appendix 4A

Results from Generalized Linear Models (GLMs) on the accuracy of parentage and sibship assignments. Best models only shown. Significance codes: '***' 0.001; '**' 0.01; '*' 0.05; 'NS' not significant. Each model table followed by AIC table for GLM selection. All tables ranked with best model first.

Table 4A.1a. Parentage assignment accuracy with all numbers of loci included.

Factor	Residual deviance in the model	Deviance explained by each term	Significance
Base	509.3	-	-
Loci	417.8	91.5	***
Sampling	247.4	170.4	***
allelicD	238.3	9.1	**
loci:allelicD	218.3	20.0	***
samp:allelicD	206.4	11.9	***

Table 4A.1b. AIC table for parentage assignment accuracy with all numbers of loci included.

Model	AIC	ΔAIC	k	ω
Loci+Sampling+AllelicD+Loci:AllelicD+Sampling:AllelicD	814.1	-	5	0.96
Loci+Sampling+AllelicD+Loci:AllelicD+Sampling:AllelicD+Loci:Sampling	821.9	7.8	6	0.02
Loci+Sampling+AllelicD+Loci:AllelicD	822.3	8.2	4	0.02
Loci+Sampling+AllelicD+Loci:AllelicD+Loci:Sampling	828.1	14.0	5	0.00
Loci+Sampling+AllelicD+Sampling:AllelicD	837.7	23.5	4	0.00
Loci+Sampling+AllelicD	852.6	38.4	3	0.00
Loci+Sampling+AllelicD+Loci:AllelicD:Sampling	854.6	40.5	4	0.00
Loci+Sampling+AllelicD+Loci:Sampling	857.6	43.4	4	0.00
Loci+Sampling+founders	860.1	46.0	3	0.00
Loci+Sampling	867.9	53.8	2	0.00
Loci+AllelicD	931.0	116.9	2	0.00
Loci+founders	949.8	135.7	2	0.00
Loci	951.7	137.6	1	0.00
Sampling	1060.1	246.0	1	0.00
AllelicD	1111.9	297.8	1	0.00
Founders	1130.6	316.5	1	0.00
Base	1133.8	319.7	0	0.00

Table 4A.1c. Parentage assignment accuracy 10-30 loci only.

Factor	Residual deviance in the model	Deviance explained by each term	Significance
Base	201.3	-	-
Loci	131.9	69.4	***
Sampling	70.6	61.4	***
allelicD	41.1	29.4	***
loci:allelicD	30.8	10.4	***

Table 4A.1d. AIC table for parentage assignment accuracy 10-30 loci only.

Model	AIC	Δ AIC	k	ω
Loci+Sampling+AllelicD+Loci:AllelicD	236.3	-	4	0.47
Loci+Sampling+AllelicD+Loci:AllelicD+Loci:Sampling	236.7	0.4	5	0.39
Loci+Sampling+AllelicD+Loci:AllelicD+Sampling:AllelicD	238.9	2.6	5	0.13
Loci+Sampling+AllelicD+Sampling:AllelicD	245.2	8.9	4	0.01
Loci+Sampling+AllelicD+Loci:Sampling	246.3	10.1	4	0.00
Loci+Sampling+AllelicD	246.8	10.5	3	0.00
Loci+Sampling	297.2	60.9	2	0.00
Loci+Sampling+Founders	299.1	62.8	3	0.00
Loci+AllelicD	327.6	91.3	2	0.00
Loci	384.7	148.5	1	0.00
Loci+Founders	385.0	148.8	2	0.00
Sampling	434.5	198.2	1	0.00
AllelicD	459.5	223.3	1	0.00
Base	506.3	270.0	0	0.00
Founders	508.3	272.0	1	0.00

Table 4A.1e. Parentage assignment accuracy 40-100 loci only.

Factor	Residual deviance in the model	Deviance explained by each term	Significance
Base	216.8	-	-
Sampling	99.2	117.6	***
Founders	88.0	11.2	***

Table 4A.1f. AIC table for parentage assignment accuracy 40-100 loci only.

Model	AIC	Δ AIC	k	ω
Founders+Sampling	438.9	-	2	0.31
Sampling+Founders	438.9	0.0	2	0.31
Founders+Sampling+Loci	440.3	1.4	3	0.16
Founders+Sampling+AllelicD	440.5	1.6	3	0.14
Founders+Sampling+Founders:Sampling	442.3	3.4	3	0.06
Founders	446.5	7.6	1	0.01
Founders+AllelicD	447.8	8.9	2	0.00
Founders+Loci	448.0	9.1	2	0.00
Sampling	449.3	10.5	1	0.00
Sampling+Loci	450.8	11.9	2	0.00
Sampling+AllelicD	451.2	12.3	2	0.00
Base	455.8	16.9	0	0.00
Loci	457.4	18.5	1	0.00
AllelicD	457.8	19.0	1	0.00

Table 4A.1g. Full sibling assignment accuracy with all numbers of loci included.

Factor	Residual deviance in the model	Deviance explained by each term	Significance
Base	339.7	-	-
Loci	294.3	45.4	***
Sampling	286.1	8.2	**
Founders	283.6	2.5	NS
Loci:Sampling	272.9	10.6	***
Loci:Founders	262.3	10.6	***

Table 4A.1h. AIC table for full sibling assignment accuracy with all numbers of loci included.

Model	AIC	ΔAIC	k	ω
Loci+Sampling+Founders+Loci:Sampling+Loci:Founders	466.9	-	5	0.76
Loci+Sampling+Founders+Loci:Sampling+Loci:Founders+Sampling:Founders	469.3	2.4	6	0.23
Loci+Sampling+Founders+Loci:samp	477.4	10.5	4	0.00
Loci+Sampling+Founders+Loci:Sampling+Sampling:Founders	478.7	11.8	5	0.00
Loci+Sampling+Founders+Loci:Founders	488.0	21.1	4	0.00
Loci+Sampling+Founders	495.6	28.7	3	0.00
Loci+Sampling+Founders+Sampling:Founders	497.6	30.7	4	0.00
Loci+Sampling	503.6	36.7	2	0.00
Loci+Sampling+AllelicD	513.2	46.3	3	0.00
Loci+AllelicD	513.2	46.3	2	0.00
Loci+Founders	513.5	46.6	2	0.00
Loci	518.9	52.1	1	0.00
Sampling	530.1	63.2	1	0.00
Founders	539.3	72.4	1	0.00
Base	546.0	79.1	0	0.00
AllelicD	548.2	81.3	1	0.00

Table 4A.1i. Full sibling assignment accuracy 10-30 loci.

Factor	Residual deviance in the model	Deviance explained by each term	Significance
Base	339.7	-	-
Loci	294.3	45.4	***
Sampling	286.0	8.2	**
Founders	283.5	2.5	NS
Loci:Sampling	272.9	10.7	***
Loci:Founders	262.3	10.6	***

Table 4A.1j. AIC table for full sibling assignment accuracy 10-30 loci.

Model	AIC	Δ AIC	k	ω
Loci+Sampling+Founders+Loci:Sampling+Loci:Founders	466.9	-	5	0.76
Loci+Sampling+Founders+Loci:Sampling+Loci:Founders+Sampling:Founders	469.3	2.4	6	0.23
Loci+Sampling+Founders+Loci:samp	477.4	10.5	4	0.00
Loci+Sampling+Founders+Loci:Sampling+Sampling:Founders	478.7	11.8	5	0.00
Loci+Sampling+Founders+Loci:Founders	488.0	21.1	4	0.00
Loci+Sampling+Founders	495.6	28.7	3	0.00
Loci+Sampling+Founders+Sampling:Founders	497.6	30.7	4	0.00
Loci+Sampling	503.6	36.7	2	0.00
Loci+Sampling+AllelicD	506.5	39.6	3	0.00
Loci+AllelicD	513.2	46.4	2	0.00
Loci+Founders	513.5	46.6	2	0.00
Loci	518.9	52.1	1	0.00
Sampling	530.1	63.2	1	0.00
Founders	539.3	72.4	1	0.00
Base	546.0	79.1	0	0.00
AllelicD	548.2	81.3	1	0.00

Appendix 4B

Breakdown of parent pair assignments illustrating occurrence of ambiguous assignments (◆= correct assignments, △= incorrect assignments and □= ambiguous assignments). Error bars are 95% confidence intervals.

Chapter 5: Inter-generational genetic erosion and extreme inbreeding depression in an introduced population of little spotted kiwi

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Abstract

Translocations are a popular conservation tool, but often involve populations being founded with small numbers of individuals, creating a population bottleneck. Such bottlenecks frequently result in a loss of genetic diversity relative to the source population and can also lead to inbreeding and inbreeding depression. We used microsatellites to examine the consequences of translocation-induced bottlenecks in populations of little spotted kiwi (*Apteryx owenii*) (LSK) founded with 40 birds (Zealandia Sanctuary) and two birds (Long Island). The identities of founders is known for both Zealandia (ZF) and Long Island (LF), and we used diagnostic genotypes based on Mendelian inheritance to further identify first generation offspring (LF1, all full siblings) and subsequent inbred generations (LF2+) among Long Island LSK. Our results show that while Zealandia LSK have retained all the allelic diversity of the source population on Kapiti Island, Long Island LSK exhibit reduced allelic diversity (-0.7 alleles per locus) and both populations exhibit continued erosion of heterozygosity over generations (-11% and -29% H_e by the ZF1+ and LF2+ generations respectively), indicating an on-going bottleneck in each site. Mean relatedness was significantly higher among Long Island than Zealandia birds, but mean inbreeding was similarly low in both populations. This disparity is due to the F2+ birds comprising only 26% of the adult population on Long Island, suggesting these highly inbred birds ($F_{PED} \geq 0.25$) do not survive to adulthood. Simulations indicate that 10-15 diploid lethal equivalents are required to produce a demographic composition similar to that seen on Long Island. This study clearly illustrates the importance of sufficient numbers of founders in translocations, the potential for on-going genetic erosion following bottlenecks, the impact that inbreeding depression can have on a population's demography and the importance of genetic monitoring to avoid the failure of conservation translocations.

Introduction

Conservation translocations (including introductions and reintroductions) have become a prevalent conservation tool as a result of habitat loss and introduced predators (Seddon 2010). Translocations to found new populations often involve small numbers of individuals for practical and financial reasons (Jamieson & Lacy 2012) and thus often result in a population bottleneck. Such bottlenecks cause reductions in allelic diversity and loss of heterozygosity, which decreases a population's ability to respond to novel environmental challenges (Allendorf 1986; Willi et al. 2006). Additionally, the small populations that result from bottlenecks are more at risk of inbreeding due to chance (Crow & Kimura 1970). Inbreeding further erodes genetic diversity and can lead to inbreeding depression: a reduction in fitness in the progeny of related versus unrelated individuals (Allendorf et al. 2013). This sequence of events has the potential to thrust a population or species into an extinction vortex; a positive feedback loop of interactions with increasingly negative impacts on the population that drive it towards extinction (Gilpin & Soule 1986).

The role of genetics in wild extinctions has been debated for decades. Species that have persisted for tens or hundreds of generations following bottlenecks (e.g., Milot et al. 2007; Johnson et al. 2009) are often cited as evidence for the harmlessness of bottlenecks. In addition it has frequently been argued that other forces are likely to drive species to extinction before inbreeding depression (Lande 1988; Young 1991; Caughley 1994; Craig 1994; Elgar & Clode 2001) and that inbreeding might actually increase fitness via purging of deleterious alleles (Templeton & Read 1984; Crnokrak & Barrett 2002). These factors, coupled with the difficulty, historically, of detecting inbreeding depression in wild populations, has led to a downplaying of the conservation importance of genetic variation and inbreeding depression (Frankham 2010). However, the post-bottleneck persistence of a handful of species does not prove that bottlenecks are not harmful (Soulé 1987; Allendorf & Ryman 2002). The impact of a bottleneck on genetic diversity is determined by the size and duration of the bottleneck. The severity of the bottleneck determines the initial loss of allelic diversity and heterozygosity and the duration of the bottleneck determines whether this loss continues over time (Nei et al. 1975). For example, heterozygosity is

expected to be lost at a rate of $1/2N_e$ per generation (where N_e = the effective population size) (Allendorf et al. 2013). Thus, populations that persist at small sizes for several generations will suffer a continual erosion of heterozygosity in each generation, prolonging the bottleneck and increasing its negative impact (Nakajima et al. 1991; Allendorf et al. 2013). Time to demographic recovery can be particularly long for populations with slow growth rates and long generation intervals (e.g., long-lived, large-bodied animals). Additionally, bottlenecks increase the likelihood of inbreeding and, thus, inbreeding depression. Evidence for inbreeding depression impacting on a variety of traits in wild populations is substantial (Frankham 2010) and inbreeding depression is an important predictor of extinction risk (Frankham 1995; Keller & Waller 2002; Frankham 2005; O'Grady et al. 2006). As a result, there have been repeated calls for genetic measures to be incorporated into conservation management and population viability analyses (PVAs) (Allendorf & Ryman 2002; Jamieson et al. 2006; Frankham 2010).

Genetic erosion and inbreeding depression have frequently been ignored in translocation monitoring because they are harder to measure and act on longer timescales than concerns such as habitat loss and introduced predators (Seddon et al. 2007; Jamieson et al. 2008). Several studies have noted a loss of genetic diversity and increased inbreeding following conservation introductions (e.g., Fredrickson et al. 2007; Brekke et al. 2010; Brekke et al. 2011) and the scale of bottlenecks during translocations is positively correlated with their likelihood of failure (Thevenon & Couvet 2002). Potential founders for translocations are rarely selected based on their lack of relatedness or genetic similarity due to logistical and financial constraints (Groombridge et al. 2012) and so may be closely related. Thus, genetic monitoring post-translocation is important to assess the necessity of supplementary translocations of genetically distinct individuals to increase genetic diversity and reduce inbreeding (e.g., Madsen et al. 2004; Heber et al. 2013). In this study, we examine the consequences of translocation-induced bottlenecks in New Zealand's little spotted kiwi (*Apteryx owenii*) (LSK).

A flightless, nocturnal ratite, LSK are the second rarest of New Zealand's five endemic kiwi species. They are long lived (27-83 years in the wild) (Robertson &

Colbourne 2004), socially monogamous and form life-long pairs (although there are instances of divorce and re-mating can occur following death of a partner). Female LSK lay one of the largest eggs relative to body size of any bird (Calder et al. 1978), but incubation (which can last up to 91 days) (Chapter 3) is carried out by the male with no involvement from the female. Little spotted kiwi were historically widespread throughout New Zealand, but were extirpated from the mainland by introduced mammalian predators by the 1980s (Holzapfel et al. 2008). The species persisted on Kapiti Island following a translocation of, at most, five birds, in 1912 (Ramstad et al. 2013). All ~1,700 extant LSK are descended from those five Kapiti Island founders and this recent, extreme bottleneck has left LSK with very low genetic diversity at both neutral and functional loci (Ramstad et al. 2010; Miller et al. 2011; Ramstad et al. 2013). Seven translocations of Kapiti Island birds to predator-free sanctuaries since the 1980s have caused secondary bottlenecks of between two and 40 individuals, resulting in reduced heterozygosity and allelic diversity of translocated populations (Ramstad et al. 2013). The magnitude of loss of genetic diversity between generations (i.e., continued loss of genetic diversity) in translocated LSK populations has not yet been tested. In spite of their lack of genetic diversity and continued conservation dependence, LSK were recently downgraded from “Vulnerable” to “Near Threatened” by the IUCN (Birdlife International 2013) and are considered to be in recovery (Miskelly et al. 2008). Here, we focus on the LSK populations founded with the highest (Zealandia Sanctuary, Wellington) and lowest (Long Island, Marlborough Sounds) numbers of birds. It is not possible to construct a pedigree for either population with the limited behavioural and genetic data available (Chapter 4).

In species or populations where mating between close relatives is likely, we might expect to see inbreeding avoidance (Blouin & Blouin 1988). For example, male Townsend voles (*Microtus townsendii*) are known to disperse further if when close female relatives are in their home range (Lambin 1994) and female superb fairy wrens (*Malurus splendens*) are more likely to engage in extra-pair fertilizations if their social mate is genetically similar to them (Tarvin et al. 2005). In LSK, where there is no possibility of dispersal for either sex due to isolation on islands and in fenced sanctuaries, inbreeding could be avoided via kin recognition and avoidance in

pairings or by extra-pair parentage (EPP) (Amos et al. 1993; Pusey & Wolf 1996). There are two mechanisms by which EPP could occur in LSK: a male could be incubating an egg that he did not fertilize (Westneat et al. 1990), or an egg that was not laid by his social mate, but was “dumped” in his nest by a different female who he may or may not have copulated with (Birkhead et al. 1990; Petrie & Møller 1991). Thus, parentage is not certain for either observed parent in LSK, but EPP has never been studied in this species. Long Island LSK were recently found to exhibit significantly lower hatching success than Zealandia LSK (Chapter 3), but it is unclear whether this is due to inbreeding depression. Here we use genetic data from LSK in Zealandia and Long Island to examine the consequences of founding populations with large (40) and small (2) numbers of individuals. Specifically, we ask:

- 1) Is there on-going genetic erosion associated with the founding of these two populations?
- 2) Is there evidence of inbreeding or inbreeding avoidance behaviours in these two populations?
- 3) Is there evidence of inbreeding depression in the Long Island LSK population?

Methods

Study sites

Zealandia is a 225ha mainland island sanctuary in Wellington, New Zealand, consisting of native podocarp and introduced pine forest. The sanctuary is surrounded by a 2.2m high fence that is impervious to the majority of introduced mammalian predators. A total of 40 LSK (22 males and 18 females) from Kapiti Island were introduced to Zealandia in 2000 and 2001. Since then, annual population growth is estimated to have been 9.5% and the current population is thought to number ~120 birds (H. Robertson, unpublished).

Long Island is a 160ha ridge of land at the northern end of the Queen Charlotte Sound in Marlborough, New Zealand. The island was cleared of mammalian predators in 1992 although an avian kiwi nest predator, the weka (*Gallirallus australis*), is occasionally present. One male and one female Kapiti Island LSK were

introduced to Long Island in 1982 and 1989 respectively and the entire Long Island population is descended from these two birds (Ramstad et al. 2013). The Long Island LSK population has also shown positive annual growth of 11.5% and the current population is believed to number ~50 birds (H. Robertson, unpublished).

Sample collection and genotyping

Six to eight pin feathers (developing feathers containing blood) were plucked (N=99 Zealandia, 38 Long Island LSK), blood drawn (N=11 Zealandia, 4 Long Island) and toe pad tissue taken (N=2 Zealandia, 1 Long Island) from a total of 112 Zealandia and 43 Long Island LSK. Handling and sampling of birds was carried out in accordance with the national Kiwi Best Practice Manual (Robertson & Colbourne 2003). Samples included the two founders of the Long Island population and 34 of the 40 Zealandia founders, all of which originated on Kapiti Island.

Genomic DNA was extracted from feather, blood and tissue samples using a proteinase K phenol–chloroform protocol (Sambrook et al. 1989), followed by ethanol precipitation. Individuals were amplified at 15 microsatellite loci for comparison of genetic diversity with a subset of the Kapiti Island birds genotyped in Ramstad et al. 2013 (excluding the Zealandia and Long Island founder birds designated as Kapiti Island birds in that analysis) and a further seven loci to give added statistical power for tests of parentage, relatedness and multi locus heterozygosity. Loci were amplified via polymerase chain reaction (PCR) in a Labnet multigene or an Eppendorf mastercycler and amplicons were visualized with universal M13 fluorescent labelling (Schuelke 2000). Loci, PCR conditions and amplicon-sizing procedures for can be found in Ramstad et al. 2010 (14 loci) and Shepherd & Lambert (2006) (*Apt59*). Seven additional loci developed for rowi were also amplified with the same PCR conditions in Ramstad et al. 2010, but with a 60°C annealing temperature (primer sequences in Ramstad et al. in prep.). Loci amplified successfully in 78% (*Rowi23*) to 100% (*Aptowe1*) of individuals assayed with a mean successful amplification rate of 96% over all loci. Repeat amplification of 15 birds (10% of final dataset) revealed a mean genotyping error rate of 0.3% across all loci.

Defining filial groups

Data were split into subsets for subsequent analyses. For Zealandia, the 34 founder genotypes were designated as founders (ZF) and all other Zealandia birds designated as non-founders (ZF1+). For Long Island, birds were designated as founders (LF), first generation, non-inbred full siblings (LF1) and their highly inbred descendants (LF2+) (see Fig. 5.1 in results). All LF2+ birds have pedigree inbreeding coefficients of 0.25 or higher (see Appendix 5A Table 5A.1 for examples) and were identified by their possession of allelic combinations that could not be observed in an LF1 individual given the genotypes of the founders and assuming Mendelian inheritance (Appendix 5A Table 5A.2). Eleven polymorphic loci provided diagnostic allele combinations that differentiated LF2+ and LF1 birds, with a 7% chance of erroneously classing an LF2+ as an LF1, and thus 93% probability of separating outbred from inbred individuals (see Appendix 5A Table 5A.2 for probability calculations). Hereafter, we refer to the ZF, ZF1+, LF, LF1 and LF2+ subsets of LSK as filial groups.

As the result of a previous study on hatching success in LSK (Chapter 3), the identities of 15 male-female social pairs of LSK in Zealandia and seven male-female social pairs on Long Island were available. These data were combined with filial group designations to examine the frequency of backcrosses in these two populations.

Measuring genetic diversity

Loci were screened for null alleles using Microchecker v2.2.3 (Van Oosterhout et al. 2004). Number of alleles and F_{IS} per locus and filial group were calculated in FSTAT v2.9.3.2 (Goudet 1995). Expected (H_e) and observed (H_o) heterozygosity per locus and filial group were estimated in GenAlEx 6.501 (Peakall & Smouse 2006, 2012). Departure from Hardy-Weinberg proportions (HWP) and gametic disequilibrium (GD) were assessed using Genepop v4.2.2 (Raymond & Rousset 1995) with sequential Bonferroni correction for multiple pairwise comparisons between loci within filial groups (Holm 1979). The *Rowi20* locus has been found to be sex-linked in another kiwi species (*A. rowi*, K. Ramstad, unpublished data). We tested for sex-linkage in all 22 loci in LSK using adult birds from both Zealandia and Long Island

for which the sex was known based on morphology (N=68). *Rowi20* had negative F_{IS} caused entirely by an excess of heterozygotes in female birds ($F_{IS} = -1.00$) and genotype correlated perfectly with sex (Appendix 5A Table 5A.3). No other loci were found to be sex-linked (data not shown). Thus, *Rowi20* was used to sex individual birds, but otherwise excluded from analyses. Calculations of multilocus heterozygosity (MLH) and all further statistical analyses were based on genotypes from 21 loci (all those available for LSK except *Rowi20*). Standardized MLH (Coltman et al. 1999) of individuals was calculated for each filial group using the package Rhh (Alho et al. 2010) in R (R Development Core Team 2013).

Assessing inbreeding and inbreeding avoidance

Shifts in MLH were investigated for ZF versus ZF1+, and LF combined with LF1 versus LF2+. Individual standardized MLH values were divided by the mean standardized MLH for ZF or LF&LF1 birds as appropriate. This resulted in a double standardized measure of heterozygosity for subsequent generations to examine the proportional loss of MLH between filial groups. All references to MLH in this study refer to double standardized heterozygosity.

The occurrence of inbreeding avoidance was investigated using data collected during nest monitoring and chick sampling over the 2011/12 and 2012/13 breeding seasons (Chapter 3). This provided genotypes for 15 putative parent-offspring triads – 10 from Zealandia and five from Long Island. To test for EPP, candidate parents were excluded as actual parents both by directly examining genotypes with the assumption of Mendelian inheritance and with a likelihood-based approach implemented in CERVUS version 3.0 (Kalinowski et al. 2007). The latter approach generated log-likelihood ratio (LOD) scores for each genotyped adult in the population being a parent of each genotyped chick in the parent-offspring triads. Individuals were noted as the most likely parents where assignment confidence was 95% confidence or greater or (where there was insufficient statistical power to assign parentage), when they had the highest LOD scores. CERVUS was also used to calculate how many other genotyped candidate parents in each site had zero mismatched loci with the chicks being tested.

Relatedness (r_{xy}) and inbreeding (F) for each population were estimated using the program COANCESTRY (Wang 2011). COANCESTRY implements two likelihood and five moment estimators to evaluate relatedness of pairs of individuals using molecular marker data. The two likelihood and two of the moment estimators can also be used to estimate individual inbreeding coefficients. Simulations were run in COANCESTRY to select the appropriate estimator for each analysis. Estimates produced by the triadic likelihood (TrioML) method were the most closely correlated with the simulated true r_{xy} (Pearson's $r=0.66$ and 0.72) and F (Pearson's $r=0.81$ and 0.85) for the Long Island and Zealandia marker sets respectively. Thus, estimates from the other six estimators were excluded from further analysis. Simulations suggested that mean population estimates of relatedness and inbreeding from COANCESTRY would be relatively reliable, but that estimates for dyads and individuals would be highly imprecise (Appendix 5A Figs. 5A.1 and 5A.2). Thus, only mean population estimates are reported here, with differences between populations tested for significance via bootstrapping over 1000 replications.

Testing for inbreeding depression

We assessed inbreeding depression in Long Island LSK in two ways. First, as inbreeding depression may be affecting hatching success of Long Island LSK (Chapter 3), we investigated the relationship between inbreeding and reproductive performance (apparent fertility (the presence of a detectable embryo) and hatching success) for inbred (LF2+) versus outbred (LF and LF1) birds using pedigree inbreeding coefficients determined diagnostic genotypes, and MLH. Second, as inbreeding depression may also be affecting survival of inbred bird and thus their recruitment into the population, we examined the demography of the Long Island population suggested by the number of individuals in each filial group in our sample. The strength of inbreeding depression in a population is measured by the number of lethal equivalents: a group of deleterious alleles that would cause death when homozygous (Allendorf et al. 2013). If differing levels of lethal equivalents are acting on individual survival at different life stages, they will alter the demographic make-up of a population. To quantify the strength of inbreeding depression occurring on Long Island, we conducted simulations to establish the number of

lethal equivalents required to produce a population with the same demographic make-up (proportion of F2+ birds) observed among Long Island LSK.

Populations were simulated in R version 3.0.1 (R Development Core Team 2013) using a modified version of an individual-based simulation model (Kardos et al. 2013). This model allows user-specification of founder number, mating system, founder genotypes, reproduction and survival rates, duration of the simulation in years and, crucially, number of lethal equivalents acting at a variety of life-stages. Lethal equivalents are entered per haploid genome and their effect on reproduction or survival is determined by the inbreeding coefficient of the simulated individual according to Morton, Crow and Muller (1956). Populations were simulated to imitate the Long Island population as closely as possible with the exception that there were no limits to the population size. Thus, simulations were started with two founders and run for 25 years. Baseline hatching success probabilities were based on known figures for the Long Island founder pair (Chapter 3) and survival probabilities were based on estimates for kiwi in predator-free environments (chick survival 0.8, juvenile survival 0.9 and adult survival 0.95) (Robertson & Colbourne 2004; Robertson et al. 2011a). Life stages were defined as chick (0 years), juvenile (1-2 years) and adult (>2 years), with individuals becoming sexually mature at 3 years of age.

As the real Long Island founder pair is known to still be alive and reproductively active more than 25 years since translocation, the founding pair in the simulations always survived to the end of the 25 year simulation run. Thus, simulated populations would never become extinct, but final populations could be composed solely of founders and F1s if inbreeding depression on subsequent generations was strong enough. Lethal equivalents were imposed on chick survival, juvenile survival and both life stages simultaneously in quantities of 2.5, 5 and 7.5 LEs per haploid genome. Simulations were repeated 50 times for each of the nine resulting scenarios. It was not possible to incorporate the effect of lethal equivalents on male reproductive success, female reproductive success and embryo survival simultaneously and so the effects of imposing lethal equivalents on reproductive output were not simulated.

Results

The number of alleles ranged from one to six per locus and from 40 (Long Island) to 53 (Zealandia) per population. This was in agreement with previous studies on this species (Ramstad et al. 2013), with the exception of an additional allele discovered in three individuals (and confirmed via reruns) on the *Aptowe23* locus in the Zealandia population. There was no evidence of null alleles for any locus in either of the two populations tested. Three loci were found to be out of HWP in the Long Island population (*Aptowe29*, *31* and *35*), but only one of the five filial groups were found to be out of HWP (LF1: $\chi^2_{(20)}=\infty$, $P<0.001$). No biologically significant GD was detected. The signal of GD was not concentrated in any filial group (3 pairs of loci in GD in ZF, 6 in ZF1+, 3 in LF1 and 3 in LF2+) or any pairs of loci, with no loci pair appearing in all four filial groups tested.

Population demography

In Zealandia, male:female (M:F) sex ratio was 1.25:1 among founders (ZF, N=34), 1.08:1 among non-founders (ZF1+, N=78) and 1.13:1 over all birds combined. Of the 15 known social pairs in Zealandia, seven were both ZF, seven were both ZF1+ and one was a male ZF with a female ZF1+. The Long Island LSK population was highly skewed in favour of first generation birds and the sex ratio in second generation birds was strongly male biased (Fig. 5.2). The M:F ratio was 1:1.45 among first generation birds (LF1, N=29) and 4.5:1 among birds from subsequent generations (LF2+, N=14). The skew towards LF1 birds is even stronger in adult birds, with 2 LF birds, 25 LF1 birds with an M:F ratio of 1:1.36 and 10 LF2+ birds (eight males and two unsexed). Thus, F2+ birds make up just 26% of the adult LSK population on Long Island and the majority (if not all) of these birds are male (Fig. 5.2). The overall M:F sex ratio of adult birds on Long Island is 1.25:1. Of the seven known social pairs on Long Island, one was the LF pair, two were both F1 birds and four were F2+ males with F1 females (Fig. 5.1).

Figure 5.1. Simplified pedigree for Long Island as represented by the eight male LSK in table 5.3 and their known relations based on diagnostic genotypes and observed pairings. Circles represent females, squares represent males and diamonds represent birds that could not be sexed. Open shapes are the founders and F1 birds and shaded shapes are F2+ birds. A=adult bird, C=chick.

Figure 5.2. Adult population composition on Long Island as represented by a sample of 38 adult birds from the island (~76% of the current total population). F2+ birds currently represent 26% of the adult population, while the majority (68%) of the adult birds on Long Island are F1s, all of which are full siblings. No female F2+ birds have yet been detected in the adult Long Island LSK population.

Genetic erosion

The Zealandia population of LSK has retained much of the genetic diversity found on Kapiti Island, but the Long Island population shows losses of both heterozygosity and allelic diversity (Table 5.1). The average number of alleles per locus in Zealandia (2.53) was the same as that seen in the Kapiti Island population and all loci remain polymorphic. The Zealandia founders show a 5.34% decrease in mean H_e compared to the Kapiti Island population, and there is a further 11.20% decrease of mean H_e in the ZF1+ birds relative to Kapiti Island. Neither generation of Zealandia birds had a mean F_{IS} that was significantly different from zero. In contrast, all three generations of Long Island birds had an average of 1.8 alleles per locus – a decrease of 0.7 alleles per locus when compared to the source population on Kapiti Island. Five loci of the 15 tested have drifted to fixation in all generations of this population. Mean H_e shows a progressive decrease in each filial group on Long Island compared to Kapiti Island. Mean H_e is reduced by 19.3% in the founders, 22.1% in the F1s and 29.0% in the F2+ birds relative to Kapiti Island LSK. Mean F_{IS} was significantly less than zero in the F1 group on Long Island ($F_{IS}=-0.391$, $P<0.001$), but was not significantly different from zero in the founders or the LF2+ birds.

Table 5.1. Erosion of genetic variation over filial groups of two LSK populations versus the Kapiti Island source population based on 15 microsatellite loci. % change in H_e and H_o was measured relative to the source population (Kapiti Island). A=number of alleles per locus. Genotypes for Kapiti Island birds came from data used in Ramstad et al 2013 and deposited in the Dryad Digital Repository (doi:10.5061/dryad.nm341).

Population	N	Mean H_e	Change H_e (%)	Mean H_o	Change H_o (%)	Mean F_{IS}	Mean A
Kapiti Island	73	0.393	-	0.419	-	-0.059	2.5
Zealandia founders (ZF)	34	0.372	-5.3	0.388	-7.4	-0.029	2.5
Zealandia non-founders (ZF1+)	78	0.349	-11.2	0.368	-12.2	-0.034	2.5
Long Island founders (LF)	2	0.317	-19.3	0.400	-4.5	0.077	1.8
Long Island F1 (LF1)	27	0.306	-22.1	0.430	+2.6	-0.391***	1.8
Long Island F2+ (LF2+)	14	0.279	-29.0	0.312	-25.5	-0.080	1.8

Significance codes: '***' 0.001; '**' 0.01; '*' 0.05

Inbreeding and inbreeding avoidance

MLH for ZF and ZF1+ overlap almost exactly, displaying only a very slight downward shift (5% on average) in the ZF1+ filial group (Fig. 5.3A)(MLH ZF: mean = 1.00, range=0.49-1.84, ZF1+: mean=0.95, range =0.37-1.59). In contrast, mean MLH was reduced by approximately 25% in the LF2+ birds on Long Island (mean=0.75, range=0.12-1.24) relative to the LF&LF1 filial groups (mean = 1.00, range=0.67-1.34) (Fig. 5.3B). These trends are mirrored in the mean H_o values for each filial group in the two populations based on 15 loci (Table 5.1).

Of the 15 parent-offspring triads available for full parentage analysis, two displayed a single locus mismatch between one or both of the observed parents and their chick (Table 5.2). Chick A1 was heterozygous at *Aptowe3* while both its observed parents were homozygous for the same allele at that locus. Chick E1 was homozygous at *Aptowe29* for an allele that was carried by its observed mother, but not by the observed father. All other triads showed no mismatches, offering no further evidence of extra-pair parentage. Both of these mismatches could be due to genotyping error, which is expected for one to two alleles (0.3%) over all loci. Analysis of all candidate parents in each population using CERVUS showed that, based on having no mismatched loci with a chick, anywhere from 2 to 100% of individuals in the relevant population were potential parents for each chick (Table 5.2). CERVUS only assigned one parent with 95% confidence; chick B1, had a female other than the observed mother assigned with 95% certainty. The observed mother was ranked as the second most likely candidate. Neither of these candidate mothers had any mismatched loci with the chick. Ranking by LOD score placed observed parents as most likely true parents in 36% of cases and ranked observed parent pairs as the most likely true parent pairs 13% of the time (Appendix 5A Table 5A.4).

Mean r_{xy} was significantly higher in the Long Island (mean r_{xy} =0.44, variance=0.05) than in the Zealandia population (mean r_{xy} =0.18, variance=0.04) ($P<0.001$), but mean F was not significantly different between the two populations (Long Island mean F = 0.08, variance=0.02; Zealandia mean F =0.10, variance=0.02; $P=0.49$).

Figure 5.3. Double standardized multilocus heterozygosity (MLH) for Zealandia and Long Island. Panels compare (A) genotyped founders (yellow) (N=34) to the non-founder descendent birds (blue) (N=78) in Zealandia and (B) the founding pair and their F1 offspring (yellow) (N=29) to the subsequent F2+ generations (blue) (N=14) on Long Island. In both cases, lines represent a normal density distribution illustrating the mean and standard deviation of the standardized MLH of each population subset. Dashed lines accompany yellow and solid lines accompany blue.

Table 5.2. Genotypic mismatches of observed parents with 15 LSK chicks in Zealandia and on Long Island, and number of possible candidate parents for each chick based on exclusion. Chicks coded with the same letter had the same putative parent pair based on nest observations.

Site	Chick	# loci mismatches with observed father	# loci mismatches with observed mother	# loci mismatches with observed parent pair	# candidate males with no mismatched loci (proportion)	# candidate females with no mismatched loci (proportion)	# pairs with no mismatched loci (proportion)
Zealandia (# candidate fathers = 46, # candidate mothers = 42, # possible pairs = 1,932)	A1	1	1	1	13 (0.28)	7 (0.17)	9 (0.00)
	B1	0	0	0	10 (0.22)	10 (0.24)	3 (0.00)
	B2	0	0	0	11 (0.24)	17 (0.40)	105 (0.05)
	C1	0	0	0	11 (0.24)	13 (0.31)	14 (0.01)
	C2	0	0	0	6 (0.13)	8 (0.19)	20 (0.01)
	D1	0	0	0	4 (0.09)	4 (0.10)	3 (0.00)
	D2	0	0	0	15 (0.33)	10 (0.24)	40 (0.02)
	E1	1	0	1	1 (0.02)	6 (0.14)	1 (0.00)
	E2	0	0	0	5 (0.11)	8 (0.19)	8 (0.00)
	F1	0	0	0	14 (0.30)	16 (0.38)	34 (0.02)
	G1	0	0	0	14 (0.70)	15 (0.94)	46 (0.14)
	H1	0	0	0	14 (0.70)	13 (0.81)	93 (0.29)
	I1	0	0	0	8 (0.40)	7 (0.44)	7 (0.02)
	I2	0	0	0	10 (0.50)	14 (0.88)	34 (0.11)
	J1	0	0	0	17 (0.85)	16 (1.00)	90 (0.28)
Long (# candidate fathers = 20, # candidate mothers = 16, # possible pairs = 320)							

Inbreeding depression

With only eight birds sampled for reproductive performance on Long Island, we did not have the statistical power to perform a regression of fitness traits against F or MLH. The data suggest no obvious correlation, however, between inbreeding and either apparent fertility or hatching success (Table 5.3). The most successful male was the founding male, L11, who hatched at least one chick per season and who also has the highest MLH of the eight males monitored. L28, an F2+ bird with the lowest MLH, was one of the least successful males. He hatched no chicks over the two study seasons and had no eggs containing detectable embryos, despite having two eggs in each nest. However, L9 showed similarly low reproductive success and had a relatively high MLH (0.95) (Table 5.3).

Table 5.3. Hatching success (HS) and apparent fertility (AF) for eight male Long Island birds monitored over two field seasons. 1 = at least one chick hatched or embryo detected, 0 = no success/no observable embryonic development. MLH = double standardized multilocus heterozygosity. F_{ped} = pedigree inbreeding coefficient based on filial group.

ID	Filial group	MLH	F_{ped}	2011/12		2012/13		Total	
				HS	AF	HS	AF	HS	AF
L11	Founder	1.12	0	1	1	1	1	2	2
L1	F1	1.01	0	1	1	1	1	2	2
L9	F1	0.95	0	0	0	0	0	0	0
L10	F1	1.01	0	0	1	0	1	0	2
L28	F2+	0.56	0.25	0	0	0	0	0	0
L14	F2+	1.01	0.25	1	1	1	1	2	2
L44	F2+	0.67	0.25	1	1	0	1	1	2
L31	F2+	0.71	0.25	1	1	0	1	1	2

Our simulations suggest that large numbers of LEs acting on chick and juvenile survival would be required to produce the proportion of F2+ birds observed among Long Island LSK (Figs. 5.2 and 5.4). With no inbreeding depression at all (0 LEs), F2+ birds would be expected to make up the vast majority (97%) of the population. The expected number of F2+ birds also exceeds that observed when the effect of inbreeding depression on survival is weak (2.5 LEs per haploid genome). Between 5 (acting on chick survival or a combination of chick and juvenile survival) and 7.5 (acting on juvenile survival) LEs per haploid genome were required to produce final populations where ~34% of individuals (including chicks) were F2+, as observed in

the Long Island population. Only one scenario, 7.5 LEs on chick survival, caused a complete absence of F2+ birds in the final population.

Figure 5.4. Mean proportion of F2+ individuals in simulated Long Island LSK populations with varying numbers of lethal equivalents imposed on chick survival (CS), juvenile survival (JS) and a combination of the two. Population composition includes chicks alive at the end of the simulation. N=50 simulations for each level of lethal equivalents. Error bars represent 95% confidence intervals. Dashed line marks value found in actual Long Island sample.

Discussion

Genetic erosion between filial groups

Zealandia LSK have retained all the known allelic diversity available in the source population of Kapiti Island. Although allelic diversity remains constant in both filial groups in this population, heterozygosity is still being lost, with a decrease in H_e of 11.2% in the non-founder birds. This is a larger loss than expected for one generation under the $1/2N_e$ rule, and likely reflects the fact that the ZF1+ contains second and third generation individuals, which will have experienced an even greater loss of heterozygosity. Over time, this loss will also reduce adaptive potential. Zealandia shows the greatest genetic diversity of any recently translocated LSK population yet studied. For example, the Tiritiri Matangi Island

(16 founders in 1993-5) and Red Mercury Island (12 founders in 1983) LSK populations both show lower allelic diversity than the Zealandia LSK and exhibit fixed loci (Ramstad et al. 2013). Clearly, 12-16 founders is not sufficient to retain even the small amount of genetic variation present in the Kapiti Island source population; future populations of LSK should be founded with no less than 40 birds.

In contrast to Zealandia, significant genetic variation has been lost in Long Island LSK both during and following their translocation bottleneck. The founding of this population with just two individuals has resulted in the fixation of a third of loci and loss of 11 alleles in total (29%) relative to the source population of Kapiti Island. This loss will undoubtedly impact on the ability of this population to respond to novel environmental challenges (Allendorf 1986; Willi et al. 2006). A large amount of heterozygosity was similarly lost when the population was founded and this has been subsequently exacerbated by continued erosion and inbreeding. The F2+ birds on Long Island have lost 29% of the H_e seen in the Kapiti Island population. The significant negative F_{IS} in the Long Island F1 group represents an excess of heterozygotes indicative of the extreme bottleneck; on average, one would expect a 25% excess of heterozygotes versus that predicted by Hardy-Weinberg proportions in the progeny of two individuals (Allendorf et al. 2013). Here, we see a 41% excess of heterozygotes in the LF1 group.

Inbreeding and inbreeding avoidance

The inbreeding coefficient of an individual represents the proportion of the genome that is identical by descent and the average loss of heterozygosity in the genome as a result (Crow & Kimura 1970). The 25% reduction in MLH seen between the F&F1 and F2+ groups in the Long Island population is what would be expected of a comparison between non-inbred ($F=0$) and highly inbred ($F=0.25$) individuals. Conversely, the overlap of MLH values between the founders and non-founders in the Zealandia population suggests that there has been little, if any inbreeding in this population. With 40 founders, inbreeding could have been caused by extreme reproductive skew in this population. This does not seem to have been the case in Zealandia, strengthening the argument that 40 is a sufficient number of founders for future LSK translocations (which is also the number recommended for North Island

brown kiwi (*A.mantelli*) (Weiser et al. 2013) especially if there are no adaptations for inbreeding avoidance in this species.

Inbreeding avoidance has never been studied in kiwi. Our data on extra-pair parentage (EPP) are inconclusive due to the low genetic variation exhibited by LSK. This results in numerous candidate parents within each population for each chick and low statistical power to assign parents via likelihood methods. For example, chick J1 on Long Island could have been fathered by 85% of the birds in the sample for this population and 100% of the females sampled could have been its mother according to the 15 microsatellites polymorphic in this population. Behavioural interactions are rarely observed for this species and the extent of encroachment into neighbouring territories and thus the opportunity for extra-pair copulations is unknown. Thus, the two chicks in Zealandia that showed genotypic mismatches with their parents could be real evidence of low levels of EPP or could simply be a result of genotyping error – a common issue in parentage studies (Kalinowski et al. 2007). Ratites in general are relatively promiscuous (Handford & Mares 1985; Crome & Moore 1990; Kimwele & Graves 2003; Moore 2007). Extra-pair paternity has also been detected at low levels in two populations of North Island brown kiwi (*A.mantelli*) (Ziesemann et al. 2006). However, this species is also known to form social groups of up to six individuals (Potter 1989; Ziesemann et al. 2006), which LSK have not been observed to do.

There is little point in an individual engaging in inbreeding avoidance when the alternative mates are equally closely related to them as their social mate (Szulkin et al. 2013) and thus inbreeding avoidance is not expected to occur when encounter rates with relatives are very low or very high (Jamieson et al. 2009), as is the case in Zealandia (low) and on Long Island (very high). Moreover, the kin recognition required for inbreeding avoidance would likely fail to evolve in species that do not regularly encounter relatives in a mating context (Kokko & Ots 2006). As a flightless bird housed exclusively occupying isolated sanctuaries, dispersal away from kin is not currently an option for LSK. However, that does not mean dispersal would not have been possible historically on the mainland. Both male and female LSK invest heavily in reproduction with one of the largest eggs in relation to body size and one

of the longest incubation periods of any bird (Calder et al. 1978). If the fitness benefits of genetic monogamy are high (e.g., increased reproductive success as seen in Azara's owl monkeys (*Aotus azarai*)) (Fernandez-Duque & Huck 2013), and dispersal was common historically, then kin recognition and EPPs may never have evolved in LSK. For the foreseeable future, inbreeding avoidance will have to be facilitated by conservation managers moving birds between populations.

Mean levels of relatedness are significantly higher on Long Island than in Zealandia. This is unsurprising, given the disparity in the number of birds used to found each population. In Zealandia, there is less chance for birds to be related or for inbreeding to occur. Conversely, on Long Island, all birds, except for the two founders, are closely related; the F1 generation consists entirely of full siblings and the F2+ birds are highly inbred. Given this, and the lack of evidence for inbreeding in the Zealandia MLH, it is initially surprising that there is no significant difference in the mean inbreeding coefficients of the two sites. This disparity is caused by an apparent lack recruitment of highly inbred F2+ birds on Long Island, resulting in a population that is highly skewed towards non-inbred F1 birds and little difference in average inbreeding between Long Island and Zealandia. If Long Island had the number of F2+ birds expected under normal population growth, then there would be a large number of birds with $F \geq 0.25$ and mean inbreeding on Long Island would be significantly higher than in Zealandia.

Inbreeding depression on Long Island

The skew towards F1 birds in the Long Island population suggests that inbreeding depression is affecting survival and, possibly, reproduction of F2+ birds in this population. Our simulation data suggests that somewhere between 10 and 15 diploid lethal equivalents acting on chick survival, juvenile survival or both would be required to produce the amount of demographic skew observed in the population. We simulated a highly simplistic scenario as inbreeding depression can affect a wide variety and combination of fitness traits including hatch rates (Briskie & Mackintosh 2004; Brekke et al. 2010), sperm motility (Fitzpatrick & Evans 2009; Malo et al. 2010; Maximini et al. 2011), parasite susceptibility (Coltman et al. 1999) and the ability to hold a territory (Seddon et al. 2004). Considering just one life history stage

underestimates genetic load (O'Grady et al. 2006; Grueber et al. 2010) and it is likely that inbreeding depression would be acting on LSK in a far more complex fashion than simulated here. Coupled with small sample size, this may also explain the apparent lack of a correlation between hatching success and inbreeding in Long Island birds. However, the modelling does demonstrate that the overall inbreeding load would need to be high to produce the demographic proportions observed on Long Island. A meta-analysis of lethal equivalents in wild animals across several life history stages is in agreement with our study and suggests that an average of 12.3 diploid lethal equivalents in populations suffering inbreeding depression (O'Grady et al. 2006).

The apparent lack of females in the F2+ birds on Long Island is intriguing, particularly as there is a roughly 50:50 sex ratio overall in the population. Two birds could not be sexed, but even if they are female, the adult F2+ group is still heavily male biased (80% males). This likely explains why the F2+ males with identified social mates are all paired with F1 females. Sex-specific inbreeding depression has been reported for a variety of species (Jamieson et al. 2003; Prugnolle et al. 2004; Charpentier et al. 2006; Rioux-Paquette et al. 2011). The majority of instances affect females more strongly than males, but the causal mechanisms are not well understood. In birds, females are the heterogametic sex and so would be more vulnerable to sex-linked recessive genes (Haldane 1922). Differences in energetic requirements due to sexual dimorphism are also known to impact on survival (Anderson et al. 1993; Coulson et al. 1999). This could negatively affect survival of female LSK, which are the larger sex in this species and produce an enormous energy-rich egg. Clearly, more data on LSK growth, development and differences between the sexes are required before this finding can be fully explained.

The overrepresentation of F1 birds on Long Island cannot be due to misrepresentative sampling. All birds were found initially with kiwi tracking dogs, which are known to detect different classes of birds in accordance with their true abundance in the population (Robertson & Fraser 2009). In addition, the long lifespan and overlapping generations of LSK mean that some F2+ birds would be expected to be older and larger than some of the younger F1s, making it unlikely that

F2+ birds are disadvantaged by competition with older, more experienced F1 birds. In addition, Long Island is not yet thought to be at carrying capacity for LSK and the population has been growing steadily for the past 30 years (Robertson & Colbourne 2008), suggesting resources and space have been available for additional birds.

The positive population growth estimates for Long Island are misleading as the population does not appear to be able to expand effectively past the F1 generation. If this is the case, then the population growth rate will slow and possibly go into decline once the founding pair in this population die or cease to reproduce. This could take decades to occur as LSK life expectancies range from 27-83 years in the wild (Robertson & Colbourne 2004). The age of the Long Island founders is not known, but the male and female are known to be at least 33 and 27 years old respectively (Robertson & Colbourne 2008) and are still producing 1-3 chicks per year (Chapter 3). Given the apparent strength of inbreeding depression among Long Island LSK, it is worrying that it has gone undetected for so long. This highlights the danger of relying on population growth estimates as a sole measure of population health and viability, especially in a long-lived species with overlapping generations.

Conclusion

Translocations remain the primary management tool for LSK and introduced predators the primary threat to their persistence (Holzapfel et al. 2008). However, LSK are also threatened by extremely low genetic diversity, which could be exacerbated by translocation management. Future populations of LSK should be founded with no fewer than 40 birds to retain genetic diversity and guard against inbreeding and inbreeding depression. Genotyping individuals being considered for translocations to ensure non-related founders would also be advisable.

Heterozygosity will continue to be eroded over generations as the population slowly recovers from the translocation-induced bottleneck. LSK are a useful empirical example of the fact that, in populations with relatively slow demographic growth, bottlenecks are not just a short-sharp loss of genetic variation – they persist over generations and can continue to erode heterozygosity for many years following a bottleneck. More data (both genetic and behavioural) is required to better evaluate kin recognition and inbreeding avoidance in LSK. We have shown that inbreeding

depression can have a massive impact on the demographics of LSK populations while remaining undetected due to the long life span and overlapping generations in this species. The Long Island LSK population is an excellent illustration of how a population can move towards extinction following a bottleneck, even when that bottleneck is the result of conservation action and the population is growing demographically. Effective genetic strategy, management, and monitoring are essential to ensure that translocated populations are likely to persist in the long-term and not spiral into an extinction vortex.

Appendix 5A

Table 5A.1. Examples of possible pedigree inbreeding coefficients (F_{PED}) for various F2+ birds on Long Island. Inbreeding coefficients calculated in R using the package kinship2 (Therneau et al. 2011). Note that F_{PED} cannot drop below 0.25 in an F2+ bird.

Conditions	Pairing	F_{PED}
No full sibling matings except in F1 generation	F1xF1	0.25
	F2xF2	0.25
	F3xF3	0.25
	F4xF4	0.25
Successive full sibling matings in each generation	F2xF2	0.375
	F3xF3	0.5
Backcrosses (no successive full sibling matings)	F1xF2	0.25
	FxF2	0.25
	F2xF3	0.25
	(F2xF3)xF1	0.25
Backcrosses with successive full sibling matings in each generation	F2xF3	0.5
	F1xF3	0.375

Table 5A.2. Calculation of power to distinguish between F1 and F2+ birds on Long Island using Mendelian inheritance. In the possible F2+ genotypes, those in bold and underlined = diagnostic genotypes not found in F1 birds. The probability of an F2+ bird possessing a diagnostic genotype is the product of the probability of the correct parent pair coming together to produce a diagnostic genotype and the probability of the diagnostic genotype itself being produced from that pairing. For example, in the first line of the table, two ab parents would be required to produce a diagnostic bb offspring. The probability of two ab parents based on the F1 genepool is $0.5 \times 0.5 = 0.25$. The probability of that parent pair producing offspring with a bb genotype is 0.25. Therefore, the probability of an F2+ bird possessing that genotype is $0.25 \times 0.25 = 0.06$. All other F2+ birds that do not have this genotype would, based on that one locus alone, be misclassified as F1 birds. Thus, the probability of an F2 being misclassified as an F1 at that locus is $1 - 0.06 = 0.94$. The probability of misclassification can be calculated in this way for all genotype combinations and the product of these probabilities give the probability that an F2+ individual would not have a diagnostic genotype at any of the loci available and thus be misdiagnosed as an F1 bird. Our power to reliably detect F2+ birds is given by one minus the probability that we would misdiagnose them as F1s.

Possible founder genotypes	Possible F1 genotypes	Possible F2+ genotypes	Probability of F2+ possessing diagnostic genotype	Per locus probability of misclassifying an F2+ as an F1	Number of loci like this	Probability of misclassifying an F2+ as an F1
ab x aa	aa ab	aa ab <u>bb</u>	0.06	0.94	8	0.60
aa x bc	ab ac	ab ac <u>aa</u> <u>bb</u> <u>cc</u> <u>bc</u>	0.50	0.50	2	0.25
aa x bb	ab	ab <u>aa</u> <u>bb</u>	0.50	0.50	1	0.50
ab x ab	aa ab bb	aa ab bb	0.00	1.00	NA	NA
Probability of error						0.07
Power						0.93

Table 5A.3. Statistics for the sex-linked *Rowi20* locus – determined using birds that had been previously sexed morphologically (bill length and body weight are the two main sex differentiated characteristics in LSK). In birds, females are the heterogametic sex (ZW) while males are homogametic (ZZ). For *Rowi20*, the negative F_{IS} is entirely caused by an excess of heterozygotes with respect to Hardy-Weinberg proportions in female LSK as all females are heterozygous. This, coupled with the allele frequencies at this locus illustrate that *Rowi20* is sex-linked.

Sex (determined by morphology)	F_{IS}	Allele frequencies		Observed genotypes		
		176	182	182/182	176/182	176/176
Males	NA	0	1.00	35	0	0
Females	-1.00	0.50	0.50	0	28	0

Table 5A.4. Results from CERVUS parentage analysis of 15 LSK chicks from Zealandia and Long Island. Based on LOD scores, only one assignment was made that was significant at the 95% confidence level. This assignment was between chick B1 (marked with a star) and a female that was not the observed mother. The observed mother has the second highest LOD score, but it was not high enough to be significant.

Site	Chick	Observed father had highest LOD score?	If no, rank of observed father by LOD score	Observed mother had highest LOD score?	If no, rank of observed mother by LOD score	Observed pair had highest LOD score?	If no, rank of observed pair by LOD score
Zealandia	A1	Y	NA	N	7	N	230
	B1*	Y	NA	N	2	N	2
	B2	N	4	N	7	N	50
	C1	Y	NA	N	4	N	2
	C2	N	4	Y	NA	N	7
	D1	Y	NA	N	4	N	3
	D2	N	2	N	4	N	10
	E1	N	6	N	3	N	320
	E2	Y	NA	Y	NA	Y	NA
	F1	Y	NA	N	5	N	5
Long	G1	Y	NA	N	3	N	4
	H1	N	5	N	3	N	22
	I1	Y	NA	Y	NA	Y	NA
	I2	N	4	N	12	N	13
	J1	N	3	N	4	N	4

Figure 5A.1. Results of simulation exercise in COANCESTRY to test for validity of relatedness coefficients estimated with the LSK microsatellite marker set. Simulated populations for relatedness testing consisted of 600 dyads spread equally across six categories of relatedness: parent-offspring ($r_{xy}=0.5$), full siblings ($r_{xy}=0.5$), half siblings/avuncular/grandparent-grandchild ($r_{xy}=0.25$), first cousins ($r_{xy}=0.125$), second cousins ($r_{xy}=0.03125$) and unrelated ($r_{xy}=0$). Allele frequencies, missing data and error rates for simulated microsatellite loci genotypes were based on empirical data for the Long Island and Zealandia LSK populations. Charts show spread of relatedness coefficients estimated by TrioML in COANCESTRY for simulated dyads in the six true relationship categories. Boxes represent the upper and lower quartiles, divided by the median. The 10 and 90 percent quartiles are depicted by lines and dots represent the outliers. Dashed horizontal lines mark true r_{xy} coefficients of 0.5 (parents-offspring (PO) and full siblings (FS)), 0.25 (half siblings/avuncular/grandparent-grandchild (HS)), 0.125 (first cousin (FC)), 0.03125 (second cousin (SC)) and 0 (unrelated (U)).

Figure 5A.2: Results of simulation exercise in COANCESTRY to test for validity of inbreeding coefficients estimated with the LSK microsatellite marker set. The simulated data set consisted of 2,100 individuals with inbreeding coefficients that varied from 0 to 1 at intervals of 0.05 and 100 individuals in each inbreeding category. Allele frequencies, missing data and error rates for simulated microsatellite loci genotypes were based on empirical data for the Long Island and Zealandia LSK populations. Regression line (solid) versus 1:1 line (dashed) for regressions of inbreeding coefficients estimated using Trioml in COANCESTRY versus true inbreeding coefficients for simulated individuals. Long Island $\beta=0.85$, $r^2=0.61$, $F=3260$, $P<0.001$. Zealandia $\beta=0.88$, $r^2=0.67$, $F=4257$, $P<0.001$.

Chapter 6: General discussion

Inbreeding depression is known to increase the extinction probability of populations and species. The overarching aim of this thesis was to investigate the occurrence of inbreeding depression in two translocated populations of little spotted kiwi (*Apteryx owenii*) (LSK). It comprises four studies that provide insight into translocation practice, the measurement of inbreeding via pedigrees and inbreeding depression in wild populations. These studies also provide new information on the reproductive potential of LSK and inform future management practice for this and similar species. Here, I review the findings of this thesis and the evidence for inbreeding depression in LSK. I also discuss the conservation implications of my findings, make management recommendations for LSK and outline future research that would extend the work conducted for this thesis.

Chapter overviews

Chapter 2 - Chick Timer™ software proves an accurate, disturbance-minimizing tool for monitoring hatching success in little spotted kiwi (*Apteryx owenii*)

LSK are challenging to monitor in the wild due to their cryptic and nocturnal nature. In Chapter 2, I field-tested Wildtech New Zealand Ltd's Chick Timer™ radio tag software for use in LSK. Chick Timer™ software was found to reliably report nightly activity, burrow exit times, incubation and hatching events for male LSK, while minimizing disturbance to nesting birds. I recommend its use in future LSK studies and suggest it should be trialled in other similar species where vital rates such as hatching success are problematic to obtain. I implemented radio tags preloaded with Chick Timer™ software in my field work for Chapter 3.

Chapter 3 - Population growth is not a suitable measure of translocation success: reduced hatching success suggests cryptic inbreeding depression in a growing translocated population of little spotted kiwi

Hatching success is an important vital rate in birds, both for assessing population health and for detecting inbreeding depression, but has never been measured in any LSK population except for Kapiti Island. Radio tracking and direct observation were used to measure hatching success in the Zealandia and Long Island LSK populations. LSK were observed to be capable of higher reproductive output than previously measured on Kapiti Island, with a high incidence of nests containing two eggs and, on Long Island, males incubating two successive clutches in a season. Hatching success was significantly higher in Zealandia than on Long Island, despite significantly higher reproductive effort by Long Island LSK. In birds monitored for both field seasons, hatching success was consistent in individuals from year to year. The high reproductive effort on Long Island suggests that the site is suitable for LSK and that energetic requirements of the birds there are being met. This, coupled with the consistency of reproductive performance of males from year to year suggests there may be a genetic component to hatching success. While both Zealandia and Long Island have shown positive population growth since their founding, the positive growth on Long Island is potentially misleading. If inbreeding is impacting on reproductive success, then the positive growth rate on Long Island could be caused by the continued reproductive success of the founding pair masking the

reproductive failure of the full sibling pairs in the first generation of this population. My results emphasise the importance of post-translocation monitoring of more than just population growth rates. They also indicate the need for a genetic assessment of both populations, a theme I returned to in Chapter 5.

Chapter 4 - Valid estimates of individual inbreeding coefficients from marker-based pedigrees are not feasible in wild populations with low allelic diversity

Detecting inbreeding depression requires error-free measures of individual inbreeding coefficients. Pedigrees are often recommended as the best way to measure inbreeding, but in LSK, as in many other species, the behavioural data required to construct a pedigree is absent. I used simulated LSK populations to investigate the possibility of reconstructing pedigrees for this species from molecular data alone by using individual genotypes in the program COLONY. I found that 80% accuracy in pedigree assignments would be required to produce unbiased estimates of individual inbreeding coefficients (F). Though unbiased, these estimates would still be relatively imprecise. When inbreeding occurred, obtaining 80% accuracy in pedigree reconstruction required complete sampling of all individuals that had ever existed in the simulated population and anywhere from 10 to 30 microsatellite loci. When complete sampling was not conducted, the maximum achievable accuracy for populations with inbreeding was ~65%, which lead to a downward bias in estimated F of up to 27%. As obtaining genetic samples for all individuals that have ever lived in any LSK population is not currently feasible, I was forced to conclude that there is currently no way to reconstruct a pedigree for LSK and that other methods for measuring inbreeding and detecting inbreeding depression must be considered for this species. I addressed these challenges in Chapter 5.

Chapter 5 - Intra-generational genetic erosion and extreme inbreeding depression in an introduced population of little spotted kiwi

Populations of LSK have been founded with anywhere from two to 40 founders, resulting in populations at greater and lesser risk of genetic erosion and inbreeding depression. I genotyped 113 Zealandia and 43 Long Island LSK and used the known identities of founders and, on Long Island, a set of diagnostic genotypes to separate

each population into subsets of generations. Zealandia LSK have retained all the allelic diversity observed in Kapiti Island LSK, but several alleles have been lost in the Long Island population and five loci have drifted to fixation. Both populations show continued loss of heterozygosity over generations, suggesting prolonged bottlenecks with a continuing impact on genetic diversity. Parentage analysis was inconclusive due to the lack of genetic variation in LSK and the number of markers currently available for this species. Finally, an analysis of the number of founders, first generation and second generation birds in the Long Island sample showed that the majority (68%) of this population is made up of first generation birds. Birds in subsequent generations appear to have a very low recruitment rate into the Long Island population, with a particular bias against females. This finding is in clear support of the hypothesis presented in the discussion section of Chapter 3; population growth on Long Island continues to be driven by the reproductive success of the founding pair, but the population cannot apparently expand beyond the first generation. Simulations conducted in this chapter suggest that, if this phenomenon is due to inbreeding depression, the effect would need to be very strong (10-15 diploid lethal equivalents acting on survival) to produce this kind of bias towards first generation birds on Long Island. I found no evidence of inbreeding in Zealandia and thus inbreeding depression is not currently a concern in this population.

Conclusion and limitations: Is there evidence of inbreeding depression in LSK?

Detecting inbreeding depression in species such as LSK is challenging due to the small sample sizes available for fitness surrogates and the difficulty of measuring inbreeding with few pedigree data and low genetic variation. The results presented in Chapters 3 and 5 of this thesis strongly suggest that there is inbreeding depression in the Long Island LSK population. There are, however, some caveats to this that are worth noting.

Although hatching success is clearly lower on Long Island than in Zealandia, I was unable to find a relationship between either pedigree inbreeding coefficient or multilocus heterozygosity and hatching success in Long Island LSK. This is partly an issue of sample size – with only eight breeding males sampled on Long Island, the

statistical power to detect anything at all was very low. However, with the strength of inbreeding depression predicted to be occurring on Long Island (Chapter 5), one might expect the relationship to be clearer. However, given that inbreeding depression can act on a variety of life stages (Grueber et al. 2010), measuring hatching success and fertility alone may not be sufficient to detect it. I was unable to measure chick or juvenile survival or take sperm samples from males in either population, both of which would potentially have been informative. Additionally, it was not possible to obtain DNA samples for all chicks, especially any that may have died before a detectable embryo was formed and so I do not have MLH values for all the chicks, which would also be informative. A complete picture of the ways that inbreeding depression may be acting on the Long Island population would require a longer term study – possibly several decades, given the long life span of LSK.

It is also important to remember that factors other than genetics can cause egg failure and low hatching success. The availability of calcium (Bidwell & Dawson 2005), the proximity of conspecific nests (Underwood & Bunce 2004), the presence of soil microbes (Wyneken et al. 1988) and the quality of nest substrate (Greenwald 2009) have all been shown to impact hatching success, with the latter two factors being particularly pertinent for a ground-nesting species such as LSK. In the course of this study, it was not possible to test for all environmental differences between Long Island and Zealandia that might have led to the disparity in hatching success seen between the two sites. A thorough environmental survey of each site was beyond the scope of this thesis, but would rule out the possibility of any differences in soil type, suitable nest-site availability or the presence of bacteria such as salmonella, for example, that could inhibit hatching success on Long Island. It is hard to imagine, however, a factor that would limit the survival and recruitment of inbred birds on Long Island versus non-inbred birds purely by chance without being connected to inbreeding depression. Thus, with the information currently available, I conclude that there is evidence of inbreeding depression on Long Island. This illustrates that translocated populations of LSK can be affected by inbreeding depression and should be managed to mitigate this risk.

It may initially seem strange that we see such strong inbreeding depression in Long Island LSK, but no apparent inbreeding depression in the Kapiti Island population, which was founded with, at most, five birds. There are several possible explanations for this. First, inbreeding depression is known to have a greater impact under stressful conditions (Keller et al. 2002) and Long Island may be stressful in a way that Kapiti Island is not (e.g., less space or resources). Inbreeding on Kapiti Island could also have been much weaker than on Long Island. With four founders rather than two, for example, first generation birds on Kapiti Island would have had half the chance of mating with a full sibling than first generation birds on Long Island and inbreeding would be slower to accumulate. Similarly, the Long Island population exhibits lower genetic diversity than Kapiti Island LSK as the result of a secondary bottleneck. This too could exacerbate the expression of recessive deleterious alleles and, thus, inbreeding depression. Finally, there is the possibility that Kapiti Island actually is being affected by as yet undetected inbreeding depression. LSK are long-lived and the ages of the original Kapiti Island founders that were translocated just over 100 years ago are unknown. It is possible that, as on Long Island, the population growth seen on Kapiti Island is a result of expansion of the first generation only or, if inbreeding has been slower to accumulate, the first and second generations. If this is the case then, just as on Long Island, it will be decades before the population shows any demographic signs of inbreeding depression. Resolving whether or not there is inbreeding depression in Kapiti Island LSK is important because it is the stronghold population for the species and the loss of this population would present a serious challenge to the viability of LSK.

Conservation implications

The results presented here for LSK emphasise several points regarding the importance of genetics in population viability and the management of conservation translocations. 1. The genetic risks of translocations can be mitigated if sufficient numbers of founders are used. 2. Post-translocation monitoring should encompass vital rates and genetic monitoring rather than just population growth to maximise the success of conservation translocations. 3. Inbreeding depression can have significant impacts on population demographics. 4. Endangerment statuses should take genetic data into account where possible.

Translocations will likely remain an important conservation tool for the foreseeable future. In New Zealand particularly, the difficulty of clearing the mainland of introduced predators means that translocations of native species to predator free islands and sanctuaries will continue to be a prominent conservation strategy. For these kinds of translocations to be successful, populations must be started with sufficient numbers of founders to retain as much genetic diversity from the source population as possible. Programs such as AlleleRetain (Weiser et al. 2012) are now available for conservation managers wishing to understand the genetic implications of using various founder numbers in translocations of a given species. Tools like this, along with measurement of genetic diversity and relatedness of potential founders (e.g., Richardson & Westerdahl 2003; Hansson & Richardson 2005b), must be implemented if managers wish to avoid loss of genetic diversity, inbreeding, and the loss of fitness that accompanies both phenomena. Adequate founder numbers also require the identification of locations that are large enough to house both the optimal number of founders and their descendants, which is a challenge in the face of increasing habitat loss. Translocations will always face practical and financial limitations and the most productive use of resources will involve moving sufficient numbers of genetically diverse founders to locations that are large enough to support them ecologically.

Comprehensive post-translocation monitoring is also often resource-limited, but equally important to translocation success. Monitoring is not simply about learning from mistakes when things go wrong; it also allows for on-going management. If monitoring is available to alert managers to potential problems, then these can be addressed before it is too late. For example, supplemental feeding has been implemented to boost reproduction and/or survival in translocated populations of Mauritius kestrel (*Falco punctatus*) (Jones et al. 1991), echo parakeet (*Psittacula eques echo*), pink pigeon (*Nesoenas mayeri*), Mauritius fody (*Foudia rubra*) (Jones & Merton 2012) and kākāpō (*Strigops habroptila*) (Elliott et al. 2006; Robertson et al. 2006). In New Zealand, an experiment with populations of South Island robins has shown that genetic diversity, juvenile survival and recruitment, sperm quality, and immunocompetence in translocated populations can all be improved via introducing individuals from other populations for genetic rescue (Heber et al. 2013).

Translocations frequently involve humans attempting to adequately assess the needs of a species and replicate its optimal habitat without all the information on the biology and life history of that species to hand, and thus will often require some form of on-going management. This means that, while it might seem preferable to leave translocated populations to their own devices, adequate monitoring is vital to their success. Genetic monitoring is just one of many tools that can be employed, but it can provide additional information on mating systems and it is necessary to ensure that future adaptive potential is maintained and inbreeding depression avoided.

The role of inbreeding depression in extinction risk, though long debated, is now widely agreed to be significant (Frankham 1995, 2005; O'Grady et al. 2006). This study adds to the growing literature of empirical examples of inbreeding depression in wild populations. It also illustrates that even when measuring individual inbreeding is challenging due to a lack of genetic diversity, low sample size and insufficiently powerful genetic markers, inbreeding depression can still be detected under certain circumstances. Diagnostic genotypes and demographic simulation approaches could prove informative for other species in similar situations. Thus, inbreeding depression can and should be investigated in populations of conservation concern, especially those that have experienced bottleneck effects.

Research on inbreeding depression in wild populations is likely to become more feasible in the future due to the advent of next generation sequencing technology. Next generation technology has already facilitated whole genome sequencing and the production of high density single nucleotide polymorphism (SNP) panels for a variety of non-human model species such as laboratory mice (*Mus musculus*) (Szatkiewicz et al. 2008; Church et al. 2009), zebra fish (*Danio rerio*) (Bowen et al. 2012), nematodes (*Caenorhabditis elegans*) (Doitsidou et al. 2010) and cattle (*Bos primigenius*) (Purfield et al. 2012) and non-model species including barnacle geese (*Branta leucopsis*) (Jonker et al. 2012), Atlantic salmon (*Salmo salar*), Bornean and Sumatran orangutans (*Pongo pygmaeus* and *P.albelii*) (Greminger et al. 2014), western lowland gorillas (*Gorilla gorilla gorilla*) (Prado-Martinez et al. 2013) and white tigers (*Panthera tigris tigris*) (Xu et al. 2013). Large SNP panels provide more

power to estimate inbreeding and relatedness coefficients directly (Santure et al. 2010; Saura et al. 2013). They also offer more power to detect heterozygosity fitness correlations caused by inbreeding depression. A recent study showed that heterozygosity measured with a panel of 14,585 SNPs explained five times more deviance in parasite infection in a wild population of harbour seals (*Phoca vitulina*) than that measured with 27 microsatellites (Hoffman et al. 2014a). The kind of power offered by large SNP panels will also facilitate marker-based pedigree reconstruction (Hauser et al. 2011).

Whole genome sequencing can also be used to look for runs of homozygosity (ROH) –regions of the genome featuring large numbers of contiguous homozygous markers that are likely to be autozygous and therefore indicative of inbreeding (Broman & Weber 1999; McQuillan et al. 2008). As ROH will be broken over time by recombination during meiosis, longer ROH are indicative of more recent inbreeding (Kirin et al. 2010), and inbreeding estimated by ROH has been shown to be highly correlated with pedigree inbreeding coefficients in both humans and cattle (McQuillan et al. 2008; Purfield et al. 2012). The use of ROH overcomes two major issues inherent in the use of pedigrees to measure inbreeding. First, variance in identity by descent in individuals with the same pedigree inbreeding coefficient is accounted for by considering genome-wide heterozygosity. Second, the assumption of unrelated, non-inbred founders of the study population can be overcome by varying the length of ROHs being considered in order to examine recent (very long runs) or more historical (shorter runs) inbreeding (Purfield et al. 2012). The majority of work using ROH to date has been on humans and cattle. However, a recent study used ROH to estimate the likely relatedness of the unknown parents of Snowflake, the famous wild-caught albino gorilla that was housed at Barcelona Zoo (Prado-Martinez et al. 2013). Using ROH, it was demonstrated that Snowflake was inbred and his parents were likely avuncular relations or half-siblings. Inbreeding is a rare occurrence in wild gorilla mating systems and was shown to be the likely cause of Snowflake's albinism (Prado-Martinez et al. 2013). The possibilities of ROH for conservation genetics studies are obvious and the technique will become more realistic as the cost of NGS decreases and more whole genome sequences for non-model species become available.

Even with the availability of new tools to measure inbreeding and detect inbreeding depression, such data will only be worthwhile if it can be incorporated into conservation strategy. One of the world's most widely used conservation resources is the IUCN Red List (IUCN 2014). The Red List guidelines do not currently explicitly include genetic data in their criteria for assigning species to threat categories, focussing instead on population size and declines or fluctuations in population size (criteria A, C and D), range restriction (criteria B) and probability of extinction over a defined time period (criteria E) (IUCN 2012). It could be argued that genetic data can be incorporated into the analysis of extinction probability under criteria E, but this misses two important points. The first is that persistence of a species over a defined time period alone is not sufficient if genetic variation is still being lost. Recommendations for preserving heterozygosity are generally 90-95% retention over 100-200 years (Soulé et al. 1986; Allendorf & Ryman 2002). As can be seen from the LSK populations studied here, heterozygosity is lost far more rapidly than this in bottlenecked populations, even when relatively large numbers of founders are used. Secondly, the IUCN stipulates persistence times for criteria E as 10-20 years or 3-5 generations (whichever is longer up to a maximum of 100 years) for "Critically Endangered" or "Endangered" species and 100 years (with no generation alternative) for species classed as "Vulnerable". The IUCN (2012) defines one generation as the average age of reproductive individuals. As genetic effects accumulate over generations, these timescales discriminate against long-lived species (O'Grady et al. 2008). Species such as LSK where the generation interval is estimated at 20-25 years (Ramstad et al. 2013) would have to show drastic reductions in population size or severe range restriction before being classed as threatened. In LSK, inbreeding depression has the potential to impact extinction probability, but not within the next 100 years. Thus, LSK remain classed as "Near Threatened", a status which severely underplays their lack of genetic potential to adapt to new environmental challenges in current populations and the possible impact of inbreeding depression if further bottlenecks occur.

The New Zealand threat classification system is similarly concerned with population sizes, trends and geographic restrictions (Townsend et al. 2008). Under this system, LSK are recognized to be conservation dependent and range restricted, but due to

their population growth over the past thirty years, are classed as “Recovering” (Miskelly et al. 2008). As previously stated, recovering numbers alone is not necessarily enough to ensure persistence. LSK have extremely low genetic diversity that continues to be eroded via translocation efforts. In the event of a disease epidemic or similar environmental challenge, LSK may not be able to survive. Additionally, if the Kapiti Island population was extirpated due to a predator incursion, LSK numbers would be reduced to ~500 birds split across seven isolated populations, all with less genetic diversity than Kapiti Island LSK. Even if immediate threats are controlled, genetic factors can still cause populations to go into decline (Jamieson et al. 2006), as evidenced by the scenario predicted in this thesis for LSK living on predator-free Long Island. Incorporating stochastic events into viability models is challenging and genetic data is by no means the only factor worth considering in species endangerment ratings. However, the genetic diversity of a species is important to its future persistence. Genetic data should be better incorporated into conservation assessment systems so that management actions can be adapted accordingly.

Management recommendations and future research

Current conservation plans for LSK involve managing the species as a metapopulation by translocating birds between extant populations (Ramstad et al. 2013). As several of these extant populations are at or approaching carrying capacity, new populations are also planned (H. Robertson, pers. comm., 14th February 2014). LSK management is restricted by the requirements of this species: predator-free locations with sufficient space for territory establishment of all birds and their descendants (estimated at 2ha per bird) (Colbourne & Robertson 1997). LSK are also considered a taonga (treasure) by the Māori (indigenous people of New Zealand) and any management action undertaken for this species requires consultation with the appropriate iwi (tribe(s)). As suitable sites for kiwi are limited, consideration of the conservation value of the Long Island LSK population is important. Long Island is a predator-free site that could be utilized as a crèche for kiwi species that have breeding programs, but only if LSK are removed from the island. Here, I outline some recommendations regarding the future management of

LSK and research questions that could be investigated as a result of my thesis findings.

Monitoring of LSK

Recommendation 1: LSK populations should be monitored using vital rates such as hatching success, and chick and juvenile survival, not just by population growth.

Justification: All populations of LSK (including Long Island) were assumed to be healthy because they all showed positive population growth. On Long Island, this population growth is the result of continued reproductive success in the founders producing a population comprised almost entirely of F1s. Due to the long lifespan of LSK, population growth is a misleading metric that masks problems occurring in later generations.

There is still much to learn regarding average reproductive rates for LSK. This study has revealed that LSK have the potential to be far more productive than previously thought and that the timing of reproduction also seems to vary between sites. Chick and juvenile survival rates have never been quantified for LSK (except via the measurement of juvenile recruitment on Kapiti Island) (Colbourne 1992). As inbreeding depression on Long Island may be affecting survival at these life stages, a study examining chick and juvenile survival (as well as dispersal behaviour, which is also unknown for this species) in several LSK populations would be valuable.

The two LSK populations featured in this study were founded with the highest and lowest numbers of individuals. Monitoring vital rates such as hatching success and reproductive effort in populations founded with an intermediate number of individuals (e.g., Red Mercury Island, N=12 and Tiritiri Matangi Island, N=16) and on Hen Island, which was founded 1988-89 with 38 birds, as well as a comparative study on Kapiti Island would provide a broader picture of reproductive rates in LSK. This baseline information would be useful in detecting abnormal reproductive rates in other LSK populations (present and future). Tools such as chick timer tags and remote sensor camera traps have been shown to facilitate LSK research and should be implemented in future monitoring of vital rates.

Recommendation 2: Pin feather samples plus a band number and sex (where possible) should be taken for every LSK captured in the field and archived at the Department of Conservation.

Justification: As many samples as possible will be required if the genetic tools arrive that allow us to reconstruct pedigrees or directly measure individual inbreeding in LSK populations. Genetic monitoring is likely to remain important for this species due to its bottleneck history and the challenges of traditional observation-based monitoring for LSK. Complete genetic sampling will continue to pay dividends in the future (see recommendation 3) and could be especially important for a large scale investigation into inbreeding in Kapiti Island LSK.

Recommendation 3: Future genetic research on LSK should focus on the development of next generation tools such as high density SNP panels, rather than increased numbers of microsatellite markers.

Justification: The very low genetic variation in LSK means that, even with a relatively large number of microsatellite markers, there is little statistical power to make inferences regarding inbreeding and relatedness in this species. High density SNP panels could be assembled for LSK or other kiwi species via genotyping by sequencing approaches such as restriction site associated DNA (RAD)-tags (Baird et al. 2008) or exon capture (Cosart et al. 2011). This would facilitate the detection of heterozygosity fitness correlations and improve the validity of marker-based estimates of inbreeding and relatedness across genus *Apteryx*. Both RAD-tag sequencing and exon capture are possible without a fully sequenced reference genome (Cosart et al. 2011; Senn et al. 2013), which is currently lacking for *Struthioniforme* birds. This approach would provide the first data of this kind for any ratite and could facilitate studies of inbreeding (and other genetic phenomena) across this order, one of the most basal extant bird lineages. Whole transcriptomes have recently been assembled for both LSK and rowi (*A. rowi*) and form the basis for a kiwi exon capture system currently under development (K. Ramstad, unpublished data).

Work to sequence the genome of several species of kiwi is currently underway (S. Edwards, pers. comm., November 2013). When this data becomes available, studies using runs of homozygosity would enable the investigation of both recent and historical inbreeding in kiwi, particularly LSK. This could be particularly useful to investigate inbreeding in Kapiti Island LSK, a relatively large population where the identities of the founders (who are almost certainly dead) are unknown, but where investigating inbreeding pre- and post the 1912 bottleneck, and inbreeding depression is important as this is the stronghold population for the species. However, whole genome sequencing approaches remain expensive and potentially unnecessary if reduced representation approaches can be used successfully (Narum et al. 2013).

Future translocations of LSK

Recommendation 1: Future populations of LSK should be founded with at least 40 birds. LSK populations should only be founded in locations large enough to accommodate 40 founders plus the potentially rapid population growth seen in Zealandia. Alternatively, LSK will have to be continually managed as a metapopulation, with birds being translocated as various populations reach capacity. To maximise genetic diversity in a new population, all founders should be from Kapiti Island. This may not always be possible, especially in the metapopulation scenario, and some populations may be founded with a mixture of Kapiti Island LSK and those from other populations. This will, however, result in the loss of heterozygosity, an effect which may intensify over several generations.

Justification: The Zealandia population of LSK has retained all the known allelic diversity of the Kapiti Island population plus a rare allele not found in any Kapiti Island birds to date (although it has still lost 11% of the heterozygosity present on Kapiti Island). 40 birds are seemingly sufficient to retain the maximum possible amount of genetic diversity present in extant LSK. However, all recently translocated populations of LSK studied to date (four out of the seven) show some decrease in genetic diversity (Ramstad et al. 2013, Chapter 5 in this thesis). A genetic survey of Hen Island LSK (which have not yet been assessed genetically) would be informative regarding the continued erosion of heterozygosity over

generations in this species. This population was founded with 38 birds in 1988-89 and would provide data that would more effectively predict loss of heterozygosity in LSK populations founded with a relatively large number of individuals over a longer time frame. We can be sure, however, that using birds other than those currently on or originally from Kapiti Island to found new populations will result in a greater reduction in genetic diversity in the new population than is necessary. The number of birds and their origin are both important considerations when founding new LSK populations. Any new populations of LSK would provide the opportunity for a study on both the establishment and persistence phases of LSK translocations, if regular access to the populations over several years were possible.

The conservation value of Long Island LSK

The LSK population on Long Island exhibits very low genetic diversity compared to other LSK populations, a high incidence of inbreeding and strong inbreeding depression. As a result, the use of Long Island LSK in future management of the species must be carefully considered and a plan for how the ~50 birds that make up this populations can be utilised in LSK conservation is important.

Option 1: Long Island LSK could form a research population for a long term study.

Research options for this population include:

- 1) Long Island LSK could be left as they are to form part of a long term study on inbreeding depression in a closed population.
- 2) LSK from Kapiti Island could be introduced as a long-term genetic rescue experiment.
- 3) There are currently three hybrids of LSK and rowi (*A.rowi*) living on Allports Island in the Marlborough Sounds. Two of these birds have been found to produce viable offspring (K. Ramstad, unpublished). Hybrid birds from Allports Island could be introduced to Long Island as a long term hybridisation and genetic rescue experiment.

Due to the long generation interval of LSK, all three of these options require a long term commitment from the Department of Conservation. Options two and three may require the removal of birds from Long Island to make space for newly translocated

birds. The destination for these removed birds should be carefully considered to avoid causing a decrease in genetic variation in the recipient population(s). LSK pairs tend to stay together long-term and Long Island pairs would produce inbred offspring. Thus, Long Island pairs should always be broken before translocation. This will not completely remove the reduction in genetic variation in the recipient population caused by introducing Long Island LSK, but it will minimise it. Due to the negative effects of introducing Long Island LSK to existing populations, translocations to zoos for use as advocacy birds may be the best course of action where possible.

Option 2: Long Island LSK could be used as test birds for potential new LSK habitats with moderate-high risk of predator incursion (e.g., Cape Kidnappers). However, LSK from other locations would also be required for such an experiment, as Long Island LSK could perish or fail to reproduce due to inbreeding related problems before being predated. A secondary issue caused by this strategy would be that if the population was successful and the Long Island LSK were left in the new location, problems with inbreeding depression would likely resurface. Thus, a long term plan to remove most (or all) Long Island LSK in the test site to a secondary destination or back to Long Island once the site had been proved suitable or otherwise, would be required. This would be logistically difficult and perhaps not the best use of resources. This plan would also require careful negotiation with the relevant iwi as Long Island LSK would essentially be being positioned as expendable.

Option 3: Single, first generation (non-inbred) Long Island birds could be translocated into other extant LSK populations at low frequency with little effect on genetic diversity to create space on Long Island for attempted genetic rescue using Kapiti Island birds. Establishing the outcome of this action would require close monitoring (see above).

Option 4: If the Department of Conservation's goal is to eventually reclaim Long Island as a location for a new kiwi population (e.g., as a crèche site for other kiwi species), then all current Long Island LSK would have to be removed to different sites. This would be a challenge given the difficulty of ensuring all current LSK had

been removed and in terms of finding suitable recipient populations for all current Long Island LSK. Again, zoos could be suitable destinations for Long Island LSK. It would also require intensive negotiation with iwi, especially the Te Atiawa Manawhenua ki te Tau Ihu Trust, who are responsible for Long Island and the kiwi that live there.

Summary

Inbreeding depression is arguably one of the oldest concepts in population genetics (Darwin 1868; Ives & Whitlock 2002), but has historically been difficult to detect in wild populations. LSK exemplify the issues of detecting inbreeding depression in wild populations of threatened species that have experienced severe bottlenecks. Sample sizes are small, pedigree data is lacking and the extremely low genetic diversity present renders tools such as microsatellite markers statistically challenged. What are conservation geneticists to do in such situations? One option is to give up, or to wait until more powerful tools become available. As conservation is generally considered a crisis discipline (Soulé 1985), inaction seems inappropriate. Alternatively, researchers can use the tools currently available to gather relevant data using creative approaches. This study not only provides evidence that inbreeding depression has the potential to severely impact the demographics of LSK populations (and other, similar species), it also advances the understanding of LSK ecology – knowledge which is imperative to the effective management of this species.

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