REVIEW ARTICLE

This article reviews the conservation genetics research that Ian Jamieson and his students and colleagues have completed over the last 12 years – research that contributed to Jamieson receiving New Zealand Ecological Society's 2012 Te Tohu Taiao Award for Ecological Excellence.

Significance of population genetics for managing small natural and reintroduced populations in New Zealand

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Abstract: Conservation biology has had a long-standing debate about the relative importance of genetic processes in increasing the risk of extinction in threatened species. We assume that priority should be given to *securing* a species from extinction by stopping significant declines in numbers and then managing the secured populations to *recovery* by creating opportunities for population growth. This two-prong approach endorses the importance of ameliorating the agents that are causing populations to decline and then understanding the genetic issues that can arise once populations become small but stable or slowly recovering. This starting point was the initiator for the research we commenced in the mid-1990s and continue to this day. The review covers six sections: (1) We identified inbreeding depression (using pedigree relatedness and molecular loci) in takahē (*Porphyrio hochstetteri*), robins (*Petroica australis and P. longipus*), and kākāpō (*Strigops habroptilus*), and both negative and positive effects of relatedness on fitness in the highly inbred Chatham Island black robins (*Petroica traversi*); (2) We found no relationship between pedigree inbreeding, molecular heterozygosity and fitness (HFC) in the genetically depauperate takahē, but we did find such effects in the more diverse robins; (3) We found that all threatened species in New Zealand had lost genetic diversity over time, compared with more recent bottlenecks associated with reintroductions; (4) *AlleleRetain* employs user-specified parameters to simulate demography, allele retention and inbreeding in animals with overlapping generations, and is particularly useful for identifying the maximum retention of allelic diversity of a reintroduced population. DOC is currently developing a website to assist with simulation in *AlleleRetain* to assist both DOC and community-led reintroductions; (5) A meta-analysis of 109 populations indicated that loss of neutral and functional diversity was correlated during prolonged bottlenecks, but overall loss of MHC diversity is 15% greater than neutral diversity. We found support for this pattern when we investigated the loss of genetic diversity from historical museum to contemporary samples in saddlebacks and robins. Further prioritising of individuals for targeted breeding on the basis of adaptive immune alleles in yellow-fronted parakeets (*Cyanoramphus auriceps*) and kākāpō may allow the maintenance of important functional genetic diversity over a species' recovery; (6) We described the diversity of toll-like receptor (TLR) genes that mediate innate immune responses in avian species, and note they may be simpler to use than the more complicated MHC immunity genes. We found evidence of episodic positive selection in the evolution of most avian TLRs, but within 10 bottlenecked species more evidence of genetic drift occurred than balancing selection at TLR loci.

Keywords: bottlenecks, genetic drift, heterozygosity, inbreeding, major histocompatability complex, neutral and functional loci, pedigree data, toll-like receptors

Debate about the causes and consequences of small populations

Although currently resolved for the most part, there has been a long-standing debate about the role and relative importance of genetic factors, such as inbreeding and genetic drift, in increasing the risk of extinction in threatened species. Once the various theories of population genetics were applied to small populations in a conservation context (Franklin 1980; Soulé 1980), there could be little doubt that inbreeding depression or random loss of genetic diversity could negatively affect small and isolated populations. However, many conservation biologists still wondered how much emphasis these genetic processes should receive relative to those that made the population small in the first place (Jamieson 2007a,b). The question that is raised is: if you manage or remove the agents that are restricting population growth (e.g. loss of primary habitat, introduced predators and pests, illegal poaching, over-harvesting) would the negative genetic consequences of small population size largely disappear?

Caughley (1994) saw small populations as being caused by the demographic agents noted above, which then makes them susceptible to genetic processes such as inbreeding (which can depress fitness among inbred individuals as a result of inbreeding depression) and loss of genetic diversity due to genetic drift (leaving small populations less able to adapt to environmental changes). Caughley argued that demographic

agents were the immediate interests of managers, but were poorly understood on theoretical grounds, while population genetics was strong in terms of theory, but had poor application in conservation biology.

In response to this influential review by Caughley, several prominent conservation geneticists argued that splitting the problem of small population into either demographic agents or genetic consequences was not helpful from a management perspective (Hedrick et al. 1996). Instead, they argued that all factors – whether demographically or genetically based – should be integrated to provide better resolution of the problems of small population biology and therefore reduce the risks of extinction. Although this argument provided important integration of genetics in managing small populations (Allendorf & Ryman 2002), specific views among current conservation geneticists remain mixed. Some researchers have maintained that inbreeding is unavoidable in small populations, and therefore it is bound to have a negative impact on threatened species and should always be managed, even if evidence of inbreeding depression is lacking (Frankham 2005; Frankham et al. 2010). In contrast, Bouzat (2010) argued against the 'detrimental paradigm of inbreeding', as its typically negative effects on fitness can be significantly reduced by the historical pattern of inbreeding, the current demography and chance events, and therefore inbreeding depression should always be measured or estimated before invoking management plans. Recent arguments have also arisen over minimum viable populations (MVP) (Traill et al. 2010; Flather et al. 2011) and the number of individuals needed to retain genetic diversity so that threatened populations can adapt to future environmental changes (Jamieson & Allendorf 2012; Frankham et al. 2014). Although the background for the genetic theory is well established, there are still controversies about how to apply it in a conservation or management context. This review will hopefully resolve some of these arguments, particularly in a New Zealand context, and highlight ways in which recent research contributions have helped bridge the gap between theory and application in the genetics of small population management.

A brief history of research on inbreeding in New Zealand's threatened species

Starting in the 1970s, New Zealand became a world leader in the eradication of introduced predators on small offshore islands, which led the way for translocation and reintroduction of many threatened species to safe sanctuaries (Saunders & Norton 2001; Jones & Merton 2012). At that time, wildlife managers did not pay much, if any, attention to the consequences of starting these reintroduced populations with small numbers of individuals, as long as the populations themselves established (Jamieson et al. 2006, 2008). By contrast, John Craig, from Auckland University, was well aware of the conservation genetics debate that was taking place, mostly in America. Craig realised the initial importance of offshore islands such as Little Barrier/Hauturu, Tiritiri Matangi, Kapiti and Codfish/Whenua Hou as safe-havens for species immediately threatened by introduced predators (Craig 1994; Craig et al. 2000). Craig, however, had also argued that while inbreeding depression might be more severe in generally large outbred populations situated on large continents or land masses, New Zealand and its many offshore islands are likely to have had a long history of small population sizes and historical inbreeding (Craig 1991, 1994). Hence, current inbreeding could be less detrimental due to a long period of inbreeding and genetic purging, where populations could recover after a period of natural selection against lethal or semi-lethal recessive alleles (Keller & Waller 2002). Craig had no evidence to support the claim of purging in New Zealand avian populations, but he put it forward as a hypothesis of how the detrimental effects of inbreeding could be avoided, therefore justifying the use of offshore islands as conservation sanctuaries. Nevertheless, overseas conservation geneticists were still critical of the hypothesis – that New Zealand's threatened species would be somehow less affected by inbreeding than species elsewhere (Jamieson et al. 2006).

As I was a PhD student with John Craig from 1983 to 1986, I was familiar with his arguments. While I was not working on translocations or conservation biology at the time, but on the social system of pūkeko (*Porphyrio porphyria*), we did co-author one paper on the lack of inbreeding avoidance behaviour in highly-related breeding groups of pūkeko (Craig & Jamieson 1988). In addition to genetic topics associated with taxonomy and systematics, four other research groups led programmes on conservation genetics of terrestrial vertebrates in New Zealand during the 1980s and 1990s: Dave Lambert at Auckland University and Massey University, Charles Daugherty at Victoria University of Wellington, Dianne Gleeson at Landcare Research and Jim Briskie at Canterbury University.

My own conservation work started with takahē (*Porphyrio hochstetteri*) in the late 1990s. I was initially interested in why takahē, which had been translocated from the Murchison Mountains in Fiordland to lowland island reserves around the North Island, had good adult survival but poor reproductive success (e.g. high egg infertility, low hatching success) relative to the source population. With students and collaborators, we conducted numerous studies on various behavioural, ecological and dietary factors that could affect fitness (see online Appendix 1), and eventually showed a weak but significant effect of inbreeding on reproductive success in island takahē (Jamieson et al. 2003). We decided to continue further analysis on the effects of inbreeding in takahē (see below), and in addition, started a long-term study of inbreeding in Stewart Island robins (*Petroica australis rakiura)* and South Island saddlebacks (*Philesturnus carunculatus*) that had been reintroduced to Ulva Island in Paterson Inlet. We monitored the survival of juveniles and adults and reproductive success of breeding pairs of all robins (11 years) and saddlebacks (7 years) living on the island, constructed pedigrees of the descendants from the founding birds, and also analysed DNA data to assess the use of molecular markers as indicators of relatedness. These data were collected along with a host of other background data on mating behaviour, nesting ecology and habitat choice of the two study species on Ulva Island and across the South Island (see Appendix 2).

In addition to the background studies noted above, our research on the impact of population genetics started with a number of comparative and review analyses focusing primarily on New Zealand birds. We first argued for a step-wise approach to managing inbreeding and loss of genetic diversity in recovery operations – to focus on genetic issues only after threatened populations were secure from severe declines due to heavy predation from introduced predators and other direct threats to extinction (Jamieson 2007a,b; and see below). We then reviewed the literature for New Zealand native species and found no support for Craig's (1991, 1994) hypothesis that

inbreeding depression is uncommon, although the number of quality studies using pedigrees was scarce and needed further work (Jamieson et al. 2006). We also reviewed the importance of genetic drift and retaining genetic diversity (as distinct from managing inbreeding per se) in threatened populations in New Zealand (Jamieson et al. 2008). These analyses and reviews on inbreeding depression and loss of genetic diversity are discussed in further detail in the following sections.

A two-staged approach to managing inbreeding and drift in small populations

As noted above, our main assumption in managing small populations, including those involved in reintroductions, was that priority should be given to (1) *securing* a species from extinction by stopping any significant decline in numbers and then (2) managing the secured population(s) to *recovery* by creating opportunities for population growth. This two-pronged approach endorses the importance of controlling or ameliorating the agents that are causing populations to decline (e.g. introduced predators) and then understanding the genetic issues that can arise once populations become small, but stable or slowly recovering (Jamieson et al. 2008; Jamieson & Allendorf 2012, 2013; Jamieson & Lacy 2012).

The two-pronged approach differs from other prioritisation programmes where resources are limited but priority is given specifically to threatened species that can reach minimum viable populations (MVP) of at least 5000 individuals (based on the need to have a genetically effective population size of 500) to avoid the consequences of genetic drift and allow populations to retain evolutionary potential in perpetuity. Otherwise, those recovery programmes that do not have a goal of reaching an MVP in the thousands should receive no further resources or funding (Trail et al. 2010; Clements et al. 2011). More recently, some of the proponents of this approach have redone their analyses and now argue that managers should set their recovery population to 10 000 individuals to avoid extinction (Frankham et al. 2014).

We have found a number of problems with using these calculated thresholds to predict extinction risk (Jamieson & Allendorf 2012, 2013). We have also noted that any attempt to double the maximum threshold from 500 to 1000 (Frankham et al. 2014) is based on a poor understanding of the role of natural selection, and any recommendation to change IUCN Red Lists is confusing and misleading (Franklin et al. 2014). In general, there are many problems associated with the use of simple rules in a complicated world – indeed there are no real thresholds of extinction for effective population sizes at 500 or 1000. Of course, large population sizes are normally better, but Frankham et al. (2014) have presented no convincing theory or new empirical result that justifies changing to larger values, where such a change would have far-reaching consequences (Franklin et al. 2014).

New Zealand has a good track record of securing populations from extinction, mainly by translocation and reintroduction to sites where predators are absent or have been eradicated or intensively controlled (McLean & Armstrong 1995; Taylor et al. 2005). Nevertheless, potential genetic problems emerge from the fact that the number of genetic founders in reintroductions tends to be very small and the carrying capacity for species on 'predator-free' sites also tends to be low. Small populations can increase the rate of inbreeding and inbreeding depression. Small populations can also be associated with genetic drift, where alleles are lost due to chance mortality, after which the population is less able to adapt to changes in the environment. I divide the rest of the review into five sections associated with our research on threatened avian populations in New Zealand:

1) Identifying incidences of inbreeding depression in threatened species and their consequences for population recovery;
2) Determining whether there is a relationship between pedigree inbreeding, molecular heterozygosity and fitness;
3) Identifying the loss of genetic diversity in threatened species and how this can be managed during translocations and reintroductions;
4) Determining whether loss of genetic diversity in bottlenecked populations appears in both neutral markers, such as microsatellite sequences, and the more complex functional genes, such as those associated with the immune system;
5) Examining the diversity of toll-like-receptor (TLR) immunity genes in bottlenecked populations.

Testing for inbreeding depression

The number of individuals introduced into protected areas is often small, as is the subsequent population itself, because of the typically restricted size of the species' restored habitat or site. We showed that island reintroductions of saddlebacks and robins (New Zealand's two most frequently translocated avian species) were very successful over the short term despite having relatively small numbers of founders (Taylor et al. 2005). Nevertheless, small founder and small final population sizes can result in population bottlenecks, which are associated with increased rates of inbreeding and loss of genetic diversity. Both inbreeding and loss of genetic diversity can have significant consequences for the long-term viability of reintroduced populations. We compared the pattern of inbreeding derived from pedigrees for four monogamous bird species (takahē, South Island saddlebacks, South Island robins and North Island robins (*Petroica longipes*)) reintroduced on two different islands, Tiritiri Matangi (220 ha) and Ulva Island (259 ha), in New Zealand (Jamieson 2011). The ability to use pedigrees was partly based on our molecular genetics research showing social and genetic monogamy in our study species (takahē: Lettink et al. 2002; Grueber & Jamieson 2008; saddlebacks and robins: Taylor et al. 2008; Townsend & Jamieson 2013a).

Although reintroduced populations founded with smaller numbers suffered more inbreeding, other factors including biased sex ratio, skewed breeding success and small carrying capacity also contributed to high levels of inbreeding. Founder genome equivalents are often used to describe the number of genetic founders assuming an equal contribution of offspring and no random loss of alleles across generations in reintroduced or captive populations. Of the 10–58 individuals released across the four reintroductions noted above, only 4–25 genetic founders (40–60%) contributed at least one living descendant and yielded 3–11 founder genome equivalents after only seven breeding seasons. This range is much less than the 20 founder genome equivalents recommended for captive breeding populations (Jamieson 2011). Recent practice has seen an increasing number of reintroductions to suitable areas that are smaller than those examined in this study. It might, therefore, be useful to develop long-term strategies and guidelines for reintroduction programmes that would minimise inbreeding and maintain genetic diversity.

Do these inbred reintroduced populations show signs of inbreeding depression? We now have evidence from five different New Zealand species for which inbreeding lowered fitness significantly, after controlling for the effects of other environmental and demographic variables. Given that the log of overall fitness is expected to decline linearly with increases in the inbreeding coefficient f, the slope of this relationship ($-B$) is used as a standardised measure of inbreeding depression for haploid individuals (Keller & Waller 2002). Inbreeding depression can be estimated using the following equation:

$$B = -\ln(S_f/S_0)/f \quad (1)$$

where S_f is the probability of survival at inbreeding level f and S_0 is the probability of survival at $f = 0$, with B equal to the number of lethal equivalents per haploid organism ($2B$ in diploid organism) (Morton et al. 1956). There are a number of ways to calculate lethal equivalents, but we provided a standard method that allows incorporation of more complicated modelling techniques and may be used across a number of circumstances other than inbreeding (Grueber et al. 2011a). This study is our most highly cited paper (147 in total; Web of Science, Oct. 2014).

We examined the effects of inbreeding across a full life-history continuum, from embryonic survival up to adulthood, and subsequent adult reproductive success, including recruitment of second-generation offspring in a number of New Zealand bird species. The first study was conducted in wild takahē by analysing pedigree and fitness data collected over 21 breeding seasons (Grueber et al. 2010). Although the effect size of inbreeding at individual life-history stages was small, inbreeding depression accumulated across multiple stages and ultimately reduced long-term fitness (i.e. successful recruitment of second-generation offspring). The estimated total lethal equivalents ($2B$) summed across all life-history stages were substantial (16.05, 95% CI 0.08–90.8) and equivalent to an 88% reduction in recruitment of second-generation offspring for closely related pairs (e.g. sib–sib pairings) relative to unrelated pairs (according to the pedigree). A history of small population size in the takahē could have contributed to partial purging of the genetic load and the low level of inbreeding depression detected at each single life-history stage. Nevertheless, our results indicate that such 'purged' populations can still exhibit substantial inbreeding depression accumulated across the species' life history (Grueber et al. 2010). Because inbreeding depression can ultimately affect the viability of small populations, our results illustrate the importance of measuring the effects of inbreeding across the full life-history continuum.

Inbreeding depression cannot always be measured across all life-history traits because data (e.g. male fertility, hatching success, offspring recruitment) are often difficult to collect in the wild. Therefore studies tend to focus on juvenile (over-winter) survival. Juvenile survival is part of a life-history pattern where variation in fitness is often detected, and which plays an important role in predicting recruitment and population growth rates. We measured inbreeding depression in juvenile survival in reintroduced populations and examined how this varied in relation to the size of the source population. The translocation of a small subset of individuals from a large, and presumably genetically diverse, source population could lead to high levels of inbreeding depression due to a high genetic load in the source population. The three closely-related study species that had very similar life-history characteristics were: (1) North Island robins reintroduced to Tiritiri Matangi Island and sourced from a large (~10 000 individuals) outbred population near Rotorua (Armstrong 1995); (2) Stewart Island robins reintroduced to Ulva Island and sourced from a moderately small population of ~300 individuals on the Stewart Island mainland (Harper 2009); and (3) Chatham Island black robins (*Petroica traversi*) reintroduced to Mangere and Rangatira islands, and sourced from a tiny source population on Little Mangere Island (9 ha) of ~30 birds (Kennedy 2009). We predicted that the magnitude of inbreeding depression would decrease as the source population became smaller creating greater historical opportunity for genetic purging.

A summary of our analysis of the magnitude of inbreeding depression on juvenile survival showed that our prediction from above was not entirely upheld (Table 1). Although the Tiritiri Matangi Island robin population sourced from the largest population showed the greatest magnitude of inbreeding depression as expected (Jamieson et al. 2007) and the Ulva Island robins that were sourced from a moderately small population showed a low level of inbreeding depression (Townsend & Jamieson 2013a), the black robin population, which was sourced from the smallest population and had the most prolonged bottleneck, exhibited an intermediate level of inbreeding depression (Kennedy et al. 2014). We also showed that the negative relationship between inbreeding and survival in black robins did not appear to be mediated by levels of ancestral inbreeding and may be attributed in part to unpurged lethal recessives (Kennedy et al. 2014). In summary, although the large difference in inbreeding depression between

Table 1. A comparison of the magnitude of inbreeding depression (measured by lethal equivalents; see text) for three closely-related robin species with similar life-history traits. Inbreeding depression in these three reintroduced island populations is predicted to decline with declining source population size because of the greater opportunity for genetic purging.

Species (length of pedigree)	Size of source population	Predicted inbreeding depression	Effect size (lethal equivalents)	References
North I. robin (12 years)	Large and outbred (~10 000)	++	8.28–13.4	Armstrong & Cassey (2007); Jamieson et al. (2007)
Stewart I. robin (10 years)	Moderate and inbred (~300)	+	0.347	Townsend & Jamieson (2013a)
Chatham I. black robin (19–22 years)	Small and highly inbred (~30)	0	6.85	Kennedy et al. (2014)

Tiritiri Matangi robins and Ulva Island robins is explained by predicted differences in genetic load, the inclusion of black robins confirms that continued high levels of contemporary inbreeding in a historically inbred population could lead to additional severe inbreeding depression.

We also obtained additional information of interest from each of these pedigree studies. Given that significant loss of fitness was only evident in highly inbred robins on Tiritiri Matangi and such individuals were relatively rare once the population expanded above 30 pairs, we concluded that inbreeding depression should have little influence on this robin population in the short term (Jamieson et al. 2007). However, without any gene flow into the Tiritiri Matangi robin population, the frequency of close inbreeding would eventually increase causing the population to slowly decline (Jamieson & Lacy 2012). Once the population did decline, the negative effects of inbreeding could be mediated by introducing new genetic stock of robins from elsewhere, which should produce genetic heterosis or increased fitness due to cross-breeding (Heber et al. 2013).

For Ulva Island robins, we obtained measures of inbreeding depression for three life-history patterns. Hatching success decreased as the inbreeding coefficient of the brood increased, with an LE ($2B$) of 2.41 (95% CI 0.198–6.71). Fledging success also declined as f increased, with LE at 1.94 (95% CI 0.108–4.65). Juvenile survival was strongly affected by the mother's age; nests laid by young inbred mothers had lower juvenile survival than those laid by older inbred mothers, but LE for juvenile survival was still close to zero (0.347, 95% CI −0.716 to 2.67). Therefore, inbreeding depression was significant for some life-history traits, but its overall magnitude compared with in North Island robins and other passerines (Laws et al. 2010; Laws & Jamieson 2011; Townsend & Jamieson 2013a) was relatively weak.

The results on inbreeding depression from the highly inbred black robin population became even more unexpected after we discovered a strong interaction between the relatedness of a breeding pair and the individual inbreeding coefficients of the parental members of the pair (Weiser et al. in press). We showed that high relatedness between members of a breeding pair improves survival of young black robins produced by the most-inbred mothers (but not the fathers), and produced the opposite effect for the least-inbred mothers (see the effects of inbreeding on juvenile survival in Fig. 1a). We demonstrated that this survival advantage cannot be attributed to demographic or ecological effects (Weiser et al. in press). We proposed that an inbreeding advantage arises when the genotype of a highly homozygous (inbred) parent is proven successful by that individual surviving to breeding age and the offspring have a high probability of inheriting a very similar (beneficial) genotype (because the parents are closely related to one another) (Fig. 1b). Our work provided the first indication that a genetic mechanism such as this 'proven-genotype advantage' may mitigate inbreeding depression (Weiser et al. in press).

We then conducted a population viability analysis for the black robin, incorporating all available information for all fitness traits, including complex effects of inbreeding that are discussed above. The population viability analysis indicated that the black robin population was expected to persist over the next 100 years (Weiser et al. in press). A strong positive effect of inbreeding on the prediction of viability was revealed when we simulated additional scenarios with fewer or no inbreeding effects: both cases showed slower population growth and lower viability than when the observed inbreeding effects were incorporated (Weiser et al. in press). We will return to the black robin example in the next section where we consider how to manage loss of diversity in a genetically depauperate species.

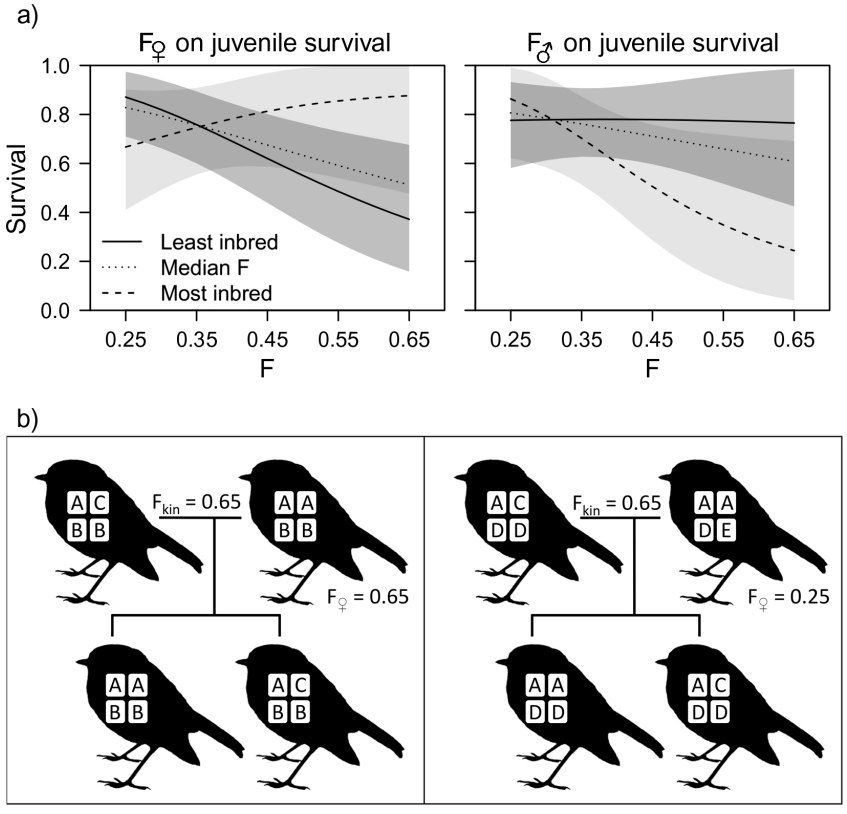

Figure 1. (a) Positive effects of highly inbred females but not males on juvenile survival in the Chatham Island black robin (*Petroica traversi*). Predictions were generated by model averaging and indicate a weighted average across subpopulations. Each line on each plot indicates survival predicted at the low (0.27), median (0.34) or high (0.54) F values of the breeding female or male. Shaded bands indicate 95% confidence intervals. (b) The positive fitness effect of inbreeding for highly inbred females, but not for less inbred birds, is explained by the "proven-genotype" advantage. For the family on the left, both parents are highly inbred, thus highly homozygous, but because they are also closely related, they share most of the same alleles. Here, further inbreeding produces offspring that are also highly homozygous and thus likely match their parents' genotype, which has proven successful by the parents' own survival to adulthood. For the family on the right, both parents are less inbred, thus less homozygous, but because they are also closely related, they share most of the same alleles. Here, further inbreeding produces offspring that are also highly homozygous, but have unproven combinations of homozygous loci, resulting in a novel genotype that may confer a low survival probability.

Decreased genome-wide heterozygosity of inbred individuals (based on neutral microsatellite markers) can be associated with reduced survival and reproductive fitness (i.e. inbreeding depression). However, showing such heterozygosity–fitness correlations (HFC) in endangered species, especially those that are already genetically impoverished, has proven to be difficult (Grueber et al. 2008a). A final study of ours has detected inbreeding depression using HFC in the highly endangered flightless parrot, the kākāpō (*Strigops habroptilus*). A recent population bottleneck of 51 individuals, a lek mating system (where only females care for eggs and young, but not the fathers) and the fact that all but one of the population's founders came from the same insular population render this species particularly susceptible to inbreeding depression (Jamieson et al. 2006; Robertson 2006). Our study used 25 microsatellite loci to derive estimates of relatedness to investigate HFC in kākāpō (White et al. in press). After accounting for the effects of co-variables, we found strong evidence that more-homozygous females laid significantly smaller clutches and had lower hatching success (White et al. in press). However, there was no evidence that male heterozygosity affected variation in egg fertility, although we did find in a concurrent study that more-homozygous males had more-abnormal sperm (White 2013; White et al. in review).

Our detection of significant HFC in kākāpō demonstrated that inbreeding depression is a contributing factor towards reduced fitness in this species. Inbreeding contributes to poor hatching success in kākāpō, which in turn reduces population growth in this highly endangered parrot. As a result of this evidence, conservation managers are experimenting with an artificial insemination programme on free-ranging kākāpō (Blanco et al. 2010), which would minimise mating between related individuals and prevent further erosion of genetic diversity. Further, the long-term goal of the kākāpō programme is to use a pedigree to actively manage breeding across a metapopulation. An assumption common to most pedigrees is that founding individuals are unrelated, but this is clearly not the case for kākāpō, as most founders originate from a small area of Stewart Island. The founders were genotyped at 25 microsatellite loci, which were used to generate relatedness estimates and assign relationships (Bergner et al. 2014). Ninety-four pedigree relationships were identified among founders comprising full-sibling and half-sibling pairs, as well as 110 with high pairwise relatedness values indicating likely close relationships. These relationships, along with mtDNA control region haplotypes, were combined to produce a new pedigree, which is currently being used to manage breeding of kākāpō in the wild.

Relationships between pedigree inbreeding, molecular loci and fitness

Although pedigrees are the best way to evaluate relatedness between individuals, they are usually unavailable in most wild populations (Pemberton 2004). As we indicated above, molecular estimates of relatedness can be used as surrogates to measure homozygosity by descent. The corresponding expected decrease in the heterozygosity of neutral markers has led to studies of the effects of inbreeding through HFC (David 1998; Hansson & Westerberg 2002; Pemberton 2004). The existence of HFC is not a new idea (Nevo 1978), but their application to detecting inbreeding depression in threatened species is a relatively recent development (Grueber et al. 2008a).

The majority of reported HFC are from large, outbred populations. While these are of interest in an evolutionary context, such results may not translate directly to threatened populations that have undergone severe bottlenecks (Grueber et al. 2008a). Bottlenecked populations exhibit increased incidence of inbreeding, increased linkage disequilibrium, reduced genetic diversity, and possible effects of historical inbreeding, such as purging. These factors may affect our ability to detect inbreeding depression in threatened species, and our interpretation of the underlying mechanisms for observed heterozygosity–fitness relationships (Grueber et al. 2008a). We are fortunate in that we have established long and detailed pedigrees in our bottlenecked study populations and these pedigrees are accompanied by molecular-derived measures of relatedness. Along with estimates of survival and reproductive success, we are in an ideal position to test whether pedigree- and molecular-derived estimates of relatedness are strongly correlated and which is the best predictor of fitness.

We conducted a study of HFC in a free-ranging pedigreed population of a severely bottlenecked species, the takahē (Grueber et al. 2011b). Pedigree-based inbreeding depression had already been detected in this species (Grueber et al. 2010; see above). Using 23 microsatellite loci developed for takahē (Grueber et al. 2008b), we observed only weak evidence of the expected relationship between multilocus heterozygosity and fitness at individual life-history stages (such as survival to hatching and fledging), and parameter estimates were imprecise (had high error). Furthermore, our molecular dataset could not accurately predict the inbreeding status of individuals (as 'inbred' or 'outbred', determined from pedigrees), nor could we show that the observed HFC were the result of genome-wide identity disequilibrium (Grueber et al. 2011b). These results may be attributed to high variance in heterozygosity within inbreeding classes (see below: Townsend & Jamieson 2013b). This is an empirical study from a free-ranging endangered species, which suggests that even relatively large numbers of microsatellite loci may provide only imprecise estimates of individual genome-wide heterozygosity, particularly in a species that has low genetic diversity (Grueber et al. 2011b).

We next assessed evidence for inbreeding depression in three life-history patterns (hatching success, fledging success, and juvenile survival) in an isolated population of Stewart Island robins, using both pedigree- and molecular-derived measures of relatedness. However, in this case the level of genetic diversity in Stewart Island robins was considerably more than that of the takahē. We found that the two pedigree and molecular estimates of relatedness were highly correlated and both supported evidence for significant but weak inbreeding depression, although evidence from pedigrees had a slightly stronger effect than those from microsatellite DNA (Townsend & Jamieson 2013a).

In a second study of Stewart Island robins, we used a full-sibling design to focus on the differences in juvenile survival associated specifically with individual sibling variation in standardised multilocus heterozygosity when expected f (from pairs of full sibs) was identical (Townsend & Jamieson 2013b). We found that within broods, siblings with higher multilocus heterozygosity at microsatellite loci experienced a higher probability of juvenile survival. This effect, however, was detected primarily within broods that experienced inbreeding or when inbreeding had occurred in their pedigree histories (i.e. at the parents' level). Thus we show, for the first time in a wild population, that the strength of an HFC is partially dependent on the presence of inbreeding events in the recent pedigree history (Townsend & Jamieson 2013b).

Loss and retention of genetic diversity in bottlenecked populations

Many threatened species that have been through severe bottlenecks appear to recover, with or without management intervention. However, such species have almost certainly lost a great deal of genetic diversity, thus making them vulnerable to new pathogens or changing environmental conditions (Amos & Balmford 2001). Previously, New Zealand's recovery programmes tended to focus on increasing population size while neglecting the importance of maintaining genetic diversity (Jamieson et al. 2006, 2008). Many threatened species have low genetic diversity and consequently may still be at risk in the long term to reduced resilience even if the effects of introduced predators are eliminated. The three main factors affecting genetic diversity – genetic drift, inbreeding, and population subdivision – are processes that potentially affect many of our locally threatened species, but these effects tend to occur over a broader timescale than ecological influences, and as such are much more difficult to detect and to justify additional resource spending. Our recent research has promoted the idea that genetic management of threatened species should not take priority over other management concerns such as controlling predators or improving habitat quality (Jamieson 2007a,b; Jamieson et al. 2008; Jamieson & Allendorf 2012; Jamieson & Lacy 2012), but genetic management needs more attention than it has received in the past. The following studies summarise our findings about loss of genetic diversity and how to manage its retention.

The New Zealand archipelago provides an ideal system to compare genetic diversity of mainland populations, where introduced predators are common, to that of smaller offshore islands, which serve as predator-free refuges. One of our first studies assessed microsatellite DNA variation in South Island robins, across large versus small mainland populations and natural versus reintroduced island populations (Boessenkool et al. 2007). Large mainland populations exhibited more polymorphic loci and higher numbers of alleles than smaller mainland and natural island populations, but genetic variation did not differ between natural and reintroduced island populations. Significant population differentiation was largely based on heterogeneity in allele frequencies (including fixation of alleles), as few unique alleles were observed (Boessenkool et al. 2007). This study showed that large mainland robin populations retain higher levels of genetic diversity than natural and reintroduced island populations. It also highlights the importance of protecting these mainland populations and potentially using them as a preferred source for new translocations.

We also compared historical and contemporary genetic variation in two threatened species, the South Island saddleback and robin, with disparate bottleneck histories (Taylor et al. 2007). Saddlebacks showed massive loss of variation in microsatellite loci when extirpated from the mainland, but no significant loss of variation following a severe bottleneck in the 1960s when the last remnant island population was reduced from ~1000 to 36 birds (Fig. 2). Low genetic variation was probably characteristic of this isolated island population and considerably more genetic variation would exist in saddlebacks today if a mainland population had survived. In contrast to saddlebacks, contemporary South Island robin populations showed only a small decrease in genetic variation

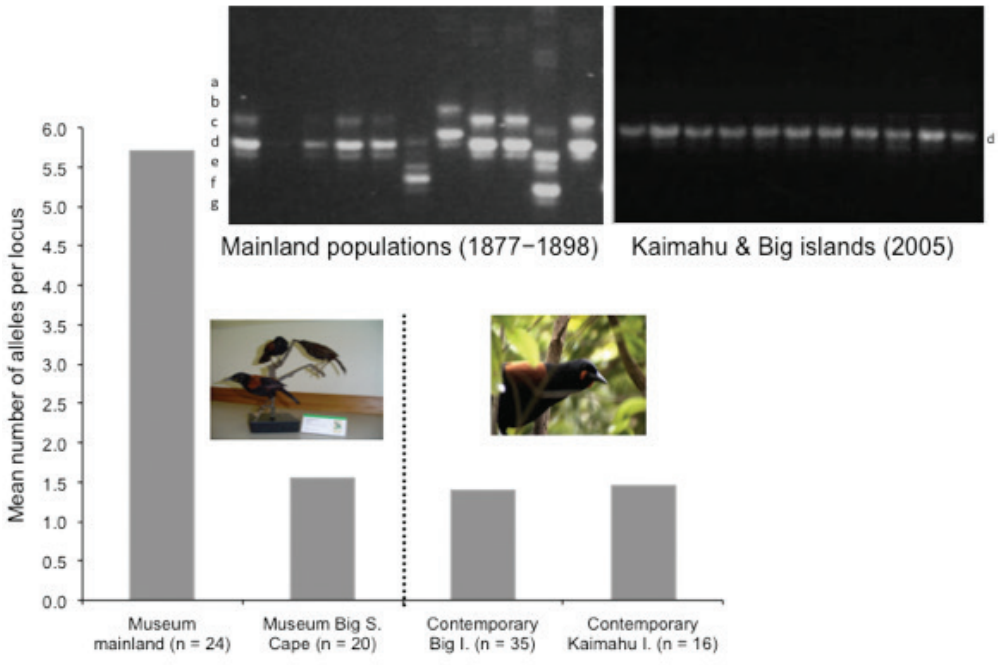

Figure 2. A comparison of genetic variation in microsatellite loci between historical and contemporary South Island saddlebacks (*Philesturnus carunculatus*). The inset shows a typical difference in variation for one microsatellite locus between the historical mainland and Big South Cape Island and the contemporary populations on Kaimahu and Big islands. Saddlebacks showed massive loss of genetic variation when extirpated from the mainland, but no significant loss of variation following a bottleneck in the 1960s when the last remnant island population (Big South Cape) was reduced from ~1000 to 36 birds, which were then released on rat-free Kaimahu and Big islands. Low genetic variation in the historical Big South Cape Island population was probably a result of the island being isolated from the mainland and having low gene-flow. Therefore the decline and recovery of South Island saddlebacks was predicted by its degree of isolation from introduced predators and not by genetic diversity.

of microsatellite loci compared with historical populations. Genetic variation was probably retained more in robins than saddlebacks because of their superior ability to fly and disperse and their ability to coexist with introduced predators, such as rats (Taylor et al. 2007). These results illustrate that contemporary genetic variation might depend more on the nature of the species and its evolutionary history than on the consequences of recent bottlenecks.

Following on from the above study, we examined the effect of repeated bottlenecks, in the form of sequential translocations, on loss of microsatellite genetic diversity in the threatened South Island saddleback. Although a slight, but non-significant loss of alleles occurred between first-order translocations and the extirpated source population, first-, second-, and third-order translocated populations had very similar levels of genetic variation to each other (Taylor & Jamieson 2008). The most obvious difference among the seven island populations appeared to lie in allele frequencies, with little or no loss of alleles among extant populations. Although sequential translocations are known to cause loss of variation in genetically diverse species, our study indicates that genetically depauperate species may be less sensitive to further loss through founder events, presumably because the few remaining alleles are well represented in founding individuals. These results show that historical bottlenecks may have a long-term effect on genetic variation, to the extent that contemporary population bottlenecks may leave no appreciable genetic signature (Taylor & Jamieson 2008). Our results suggest that subjecting genetically depauperate endangered species like saddlebacks to sequential translocations could be used to rapidly establish new populations without further eroding genetic variation. The results for robins and saddlebacks means that background genetic diversity of the species cannot necessarily be generalised.

Between the early 1980s and 1990s, 25 takahē (mostly juvenile) were translocated from the sole remnant population in Murchison Mountains, Fiordland, to four offshore islands from which introduced predators had been eradicated (Jamieson & Ryan 2000, 2001). The subsequent island sites were closely monitored for up to seven generations since founding (Grueber & Jamieson 2008). Gene-drop analysis, based on 31 genetic founders from Fiordland, indicated that 7.5% of genetic diversity on the islands had been lost over the relatively short time-frame due to both a failure to equalise founder representation early on and subsequent disproportionate breeding success (founder genome equivalents = 6.6). Predictions from pedigree modelling suggest that 90% of genetic diversity will be maintained for only 12 years, but by introducing a low number of immigrants from Fiordland and permitting the population to grow, 90% of genetic diversity could be maintained over the next 100 years (Grueber & Jamieson 2008).

Although the above recommendation was applied to the overall island population of takahē, in the last three years the capacity of island populations in the North Island has increased substantially with the recent introduction of takahē to Motutapu Island (1500 ha) in the Hauraki Gulf. We are currently working with the Takahe Recovery Group to see whether the future estimated size of the North Island population could be large enough to sustain a separate population from the South Island birds (Grueber et al. 2012). Why would we want to do this? Although takahē were once widespread throughout New Zealand, two different (flightless) species evolved in the South Island (*Porphyrio hochstetteri*) and North Island (*P. mantelli*), possibly established by separate founding events from Australia (Trewick 1996, 1997). These two species were not only distinct genetically, but also morphologically, with fossil evidence indicating that the North Island *P. mantelli* had a smaller beak, longer and more slender leg bones and lighter body weight than the South Island's *P. hochstetteri* (Trewick & Worthy 2001). Therefore, the current takahē recovery programme has facilitated the introduction of an ecological replacement for a now extinct species in the North Island in order to secure the last remaining natural wild population in the Murchison Mountains, South Island. The geographical distribution of these new populations was simply a consequence of coastal islands with patches of (introduced) grassland habitat, and no introduced predators, being more available and accessible around the North Island and the Marlborough Sounds (Maud Island) (Jamieson & Ryan 2000). The current geographic pattern is maintained by gene flow from the South Island, which will prevent or slow local adaptations to northern island environments from occurring. Therefore, the key question is whether future population sizes can become large enough to retain genetic diversity within both North Island and South Island metapopulations (Jamieson 2013).

Genetic structure across fragmented populations

Contemporary patterns of genetic structure among fragmented populations can either result from natural barriers inhibiting gene flow (e.g. mountain ranges, large bodies of water) or from human-induced fragmentation of habitat. Use of historical samples collected prior to fragmentation allows for the origin of genetic structure to be established. We compared historical and contemporary levels of genetic diversity and structure of an endangered passerine, the mohua or yellowhead (*Mohoua ochrocephala*), using microsatellite DNA (Tracy & Jamieson 2011). We found that significant allelic richness in mohua had been lost over the last 100 years. Close to half of this was due to extinction of birds from entire regions, but almost as much was due to loss of genetic diversity within extant populations. We also found that minimal genetic structure existed historically when the habitat was more continuous. We concluded that the genetic structure apparent today in mohua resulted from anthropogenic effects of recent fragmentation and isolation (Tracy & Jamieson 2011).

Analysis of historical takahē samples tells a different story. We used 20 microsatellite markers to genotype samples of the three existing takahe museum specimens (1849–1898) to estimate levels of genetic diversity and effective population size (Grueber & Jamieson 2011). These estimates were compared with equivalent estimates from DNA samples of three specimens preserved at the time of rediscovery (1949), and to 20 contemporary samples. Using rarefaction simulations to account for limited sample sizes, results suggest that only slightly more genetic diversity (allelic diversity and numbers of polymorphic loci) existed in the earliest takahē sampled, and that levels of genetic diversity at the time of rediscovery were very similar to contemporary observations.

Contemporary samples from a widespread congener to takahē, the pūkeko, showed consistently higher levels of genetic diversity and greater effective population size, even after rarefaction equivalent to the small sample sizes available for takahē. It is likely that the population size of takahē in Fiordland at the time of European arrival in the 1800s was similar to

its current size (Grueber & Jamieson 2011). Our molecular results indicate a relatively small and isolated population in Fiordland where takahē may never have been very common.

These examples emphasise the importance of assessing genetic structure of populations prior to their fragmentation. This also highlights the growing significance of museum specimens as a tool in understanding genetic diversity and phylogeography of threatened species (e.g. yellow-eyed penguin, *Megadyptes antipodes* (Boessenkool et al. 2009); kea, *Nestor notabilis* (Dussex 2013); kākāpō (Bergner 2013)).

Using demographic modelling to predict allele retention

Reintroduction guidelines recommend that adequate numbers of individuals be released to minimise loss of genetic diversity, but these numbers are rarely quantified, partly because allele retention is difficult to predict beyond one generation for real populations with complex demography and life-history traits. Alleles that are lost can only be replaced by immigration, if there is some connectivity with another population of the same species, or by mutation over a very long timespan, which is not feasible for species under immediate threat. Maximising allele retention is therefore critical for threatened populations, especially to enhance disease resistance. Heterozygosity, which influences individual fitness, is also important to consider, but even extreme bottlenecks can result in little loss of heterozygosity if the bottleneck is temporary (Allendorf 1986). We developed a framework and a computer model for assessing the number of individuals required for small population management or an island reintroduction, which takes account of allele loss during both the founding event and in the subsequent establishment phase (Tracy et al. 2011; Weiser et al. 2012). This is the first attempt to model release numbers for reintroductions in order to preserve alleles with a specified initial frequency, while taking into account variation due to post-release mortality rates, population growth rates, and site carrying capacity.

Management action may be used to improve allele retention by minimising the size of any necessary bottleneck, maximising carrying capacity, and enabling immigration. We first developed the model based on translocations of mohua and found that under typical demographic conditions, approximately 60 individuals would need to be released to achieve at least 95% certainty that alleles at an initial frequency of 0.05 would be retained after 20 years (five overlapping generations). This is double the number typically released in translocations of mohua and other threatened forest passerines in New Zealand (Tracy et al. 2011).

Other computer models that simulate allele frequency in the source population, number of founders, reproductive success and variance, and frequency of immigration can be used to predict retention of rare alleles, but these programmes are subject to limitations and are not sufficient for many in situ conservation programmes; e.g. *Mohuasim* (Tracy et al. 2011) is specific to one species. We developed a new model, *AlleleRetain*, to address these limitations (Weiser et al. 2012). *AlleleRetain* is implemented in R and freely available via its website (https://sites.google.com/site/alleleretain/) and the Comprehensive R Archive Network (cran.r-project.org). *AlleleRetain* employs a series of user-specified parameters to realistically simulate demography, allele retention, and inbreeding accumulation in animals with overlapping generations and a wide variety of life-history traits under many management options (Fig. 3). *AlleleRetain* is particularly useful for identifying the maximum retention of allelic diversity of a reintroduced population, especially when the population is capped at a small size with no natural immigration and thus at risk of genetic drift. The model simulates top-up translocations (to supplement the initial founder population in subsequent years) and immigration according to flexible specifications,

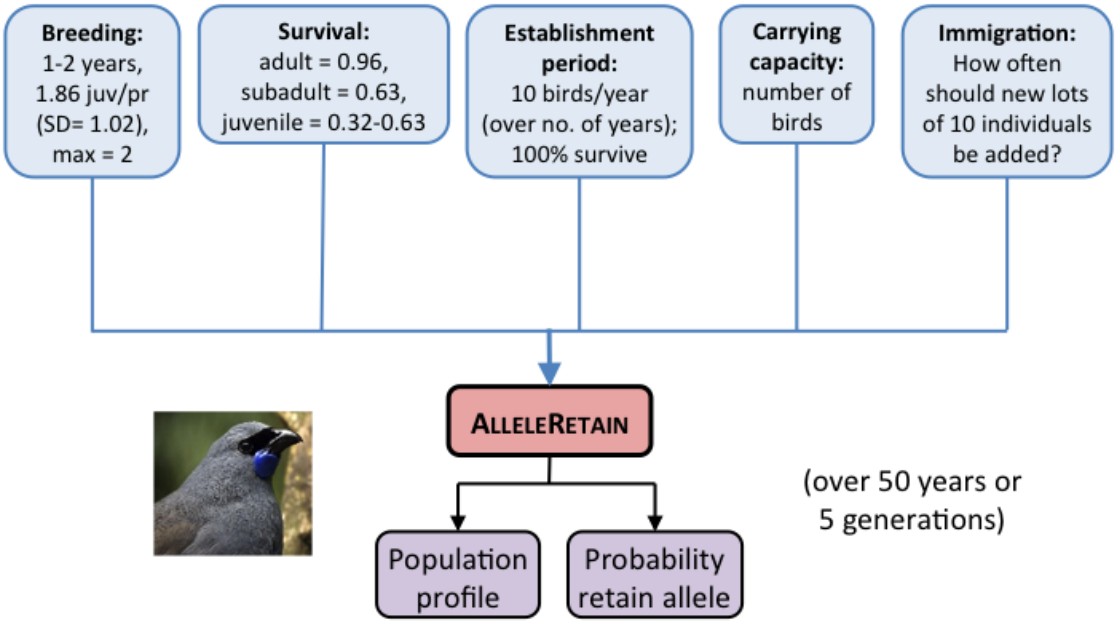

Figure 3. *AlleleRetain* is an individual-based model that simulates allele frequency, population growth, and levels of inbreeding for assessing management options for conserving allelic diversity in small natural or reintroduced populations. The diagram illustrates some of the input parameters associated with kōkako (*Callaeus cinerea*) populations that *AlleleRetain* can use to estimate the population profile and probability of loss of alleles (at a designated frequency).

and estimates the number of effective immigrants (those that breed) each generation (Weiser et al. 2012). *AlleleRetain* can also track descendant pedigrees and output the mean inbreeding coefficient of the simulated population alongside the probability of retaining rare alleles at the specified initial frequency (the only free program to do so, to our knowledge).

For specific examples of using *AlleleRetain*, see Weiser et al. (2013) and Reynolds et al. (2013). The use of *AlleleRetain* has also been documented in unpublished reports for the reintroduction of brown kiwi (*Apteryx mantelli*) to Rotokare Scenic Reserve (Weiser et al. 2011) and Haast tokoeka kiwi (*A. australis* 'Haast') in two small islands (Pomona I., Rarotoka I.) and in a fenced mainland sanctuary (Orokonui Ecosanctuary) (Weiser & Jamieson 2012). In addition, the North Island Kokako Recovery Group has been dealing with a fragmented distribution of small populations, plus requests to establish additional reintroductions from community groups. After conducting analyses for establishing and maintaining genetically robust populations across their range (Weiser 2014b), the Kokako Recovery Group is in the process of reprioritising its recovery programme.

The Department of Conservation (DOC) deals with many applications for reintroductions each year, including increasing numbers from community groups, and the most frequent query they receive is: How many individuals should be transferred and released? DOC is interested in using *AlleleRetain* for assessing the optimum number of individuals to release and/or to future-manage subsequent immigration rates in isolated populations due for reintroductions. Because *AlleleRetain* is likely to be too complicated for most community trusts to operate, DOC is in the process of setting up a website where demographic information can be submitted for particular species and transfers and then simulations run to estimate numbers for release (L. Adams, pers. comm., DOC, Wellington).

As a final example, we go back to the well-studied black robin, which underwent a genetic bottleneck of a single breeding pair over 10 generations ago (Kennedy 2009). This species raised the question of whether it is worthwhile worrying about retaining genetic diversity in a population that has undergone such a severe bottleneck. After simulating the amount of allelic diversity expected to be present in the black robin, we used a population viability analysis framework to assess management options to help retain the remaining diversity in both extant populations (Weiser 2014a). We found that a small amount of management effort (translocating one bird between populations every 2–10 years) would restore allelic diversity already lost by the smaller of the two extant populations, and would maintain >90% of any unique founder alleles in both populations over the next 100 years. We also evaluated options for establishing an additional population; for example, a population established by releasing 40 birds and reaching a carrying capacity of 400 would self-sustain allelic diversity (Weiser 2014a). These results show that species that have lost a great deal of genetic diversity may still benefit from genetic management.

Is variation between neutral and functional gene diversity correlated?

In examining the effects of population bottlenecks on loss of genetic diversity, our research, and that of others, has been mostly confined to the use of neutral DNA such as microsatellite loci. Many researchers argue that functional genes associated with the immune system can be under intense selection pressure and may not be subject to the process of genetic drift in the same way as neutral loci. For example, there is a strong association between disease resistance/susceptibility and diversity genes of the vertebrate major histocompatibility complex (MHC), which encode cell-surface proteins that are responsible for recognising and binding foreign peptides. There are numerous examples of specific MHC genotypes associated with either disease resistance or susceptibility in wild populations (Sommer 2005). Despite more than 15 years of research, single empirical studies offer highly contradictory explanations of the relative roles of different evolutionary forces – selection and genetic drift – acting on MHC genes during population bottlenecks.

In our first study involving immunity genes, we conducted a meta-analysis of 109 population comparisons to show that there was a strongly correlated loss between neutral and functional diversity during prolonged bottleneck events, but overall loss of MHC variation is 15% greater than neutral diversity (Sutton et al. 2011). To date, most conservation genetics research has relied on relatively inexpensive and putatively neutral markers, such as microsatellites, as proxies for genome-wide diversity. Our meta-analysis indicates that loss of microsatellite diversity was strongly correlated with losses of MHC – indeed the 15% greater loss of adaptive MHC variation is likely to be biologically significant and poses a concern for the management of bottlenecked populations.

Our empirical studies in New Zealand have also found support for this general pattern when we investigated the loss of genetic diversity from historical museum to contemporary samples in both functional MHC immunity genes and in neutral microsatellite DNA in bottlenecked populations of North and South Island saddlebacks and robins (Sutton et al. 2013; in review). Using a Bayesian framework, we found that the greatest losses in genetic diversity occurred for the most severely bottlenecked populations, particularly between museum mainland and contemporary samples. Additionally, where loss of diversity occurred, the change was greater for MHC genes compared with microsatellite loci (Sutton et al. in review). These results indicate that contemporary MHC variation in bottlenecked populations is shaped more by genetic drift than balancing selection. Additional New Zealand studies on black robin (Miller & Lambert 2004), tuatara (*Sphenodon* spp.) (Miller et al. 2010) and little spotted kiwi (*Apteryx owenii*) (Miller et al. 2011) have shown similar losses of MHC diversity in bottlenecked populations.

Many studies have found associations between particular host MHC genotypes and disease resistance/susceptibility (reviewed in Acevedo-Whitehouse & Cunningham 2006; Piertney & Oliver 2006). In New Zealand, we compared MHC class II B and microsatellite diversity in North Island saddlebacks from Mokoia Island that had previously been tested for prevalence of avian malaria (Sutton 2013). Although genetic structuring was observed at MHC loci and not at 19 neutral microsatellite loci, we found no evidence of particular MHC alleles being associated with malaria prevalence. As an inability to detect associations between host genetics and malaria may be due to the lack of disease intensity data (i.e. we relied solely on prevalence data), we emphasise the value of intensity data and long-term datasets for improved understanding of interactions between host genetics and disease (Sutton 2013). Our additional studies designed MHC primers for red-crowned parakeet (*Cyanoramphus novaezelandiae*) (Knafler & Jamieson 2014) and kākāpō (Knafler et al. 2014), and will facilitate the study of MHC diversity in relation to

beak and feather disease virus, which has been recently detected in wild parrot populations.

Research on MHC diversity may also be important in determining whether remnant source populations differ in their diversity of immunity genes, especially when they are currently managed as a single recovering population. Kākāpō were historically abundant on the mainland, but over several hundred years were reduced to a single adult male (Richard Henry) and 62 other individuals from Stewart Island; the population now totals 123 adults (Knafler et al. in review). We found little diversity within and across the two source areas of kākāpō for five toll-like-receptor (TLR) immunity gene loci (see below), but we did find unique MHC class II B alleles in Richard Henry (now deceased) and one of his three offspring. This result indicates that prioritising specific individuals for targeted breeding based on adaptive immune alleles may be a temporary solution to ensure the maintenance of important functional genetic diversity over the course of kākāpō recovery (Knafler et al. in review).

Diversity of toll-like-receptor (TLR) immunity genes in bottlenecked populations

Studies of functional diversity in non-model vertebrate species have been dominated by examination of MHC (see above), and are informative with respect to individual and population viability due to their direct association with immune function (Sommer 2005; Piertney & Oliver 2006). However, the high level of diversity that MHC exhibits due to gene duplications can challenge studies in non-model organisms because co-amplifying loci interfere with haplotype phasing and genetic diversity estimates (Babik 2010). Furthermore, at least half of the genetic basis of interindividual variability in immune responses to pathogens in humans is thought to be a consequence of non-MHC genes (Jepson et al. 1997; Vinkler et al. 2009). It is becoming obvious that MHC alone is insufficient to fully understand wildlife immunogenetics (Acevedo-Whitehouse & Cunningham 2006).

The toll-like receptor (TLR) genes are an ancient part of the genome of many vertebrates that mediate innate immune responses via recognition of pathogen-associated molecular patterns (PAMPs) such as double-stranded RNA of some viruses, or lipopolysaccharide of Gram-negative bacteria (Roach et al. 2005; Brownlie & Allan 2011). As a result of PAMP binding, TLRs initiate intracellular signalling responses, stimulating genes associated with inflammation and immunity, including those of MHC (Akira et al. 2001; Cormican et al. 2009) (Fig. 4). The genomes of mammals contain a suite of TLR genes that facilitate recognition of a variety of different PAMPs, but much less is known about the function of TLRs in birds (Alcaide & Edwards 2011). Growing evidence from studies of model organisms has indicated that variation in binding regions of TLR loci may reflect the potential of populations to adapt to changing environments on evolutionary timescales (Akira et al. 2001; Vinkler et al. 2009). As such, positive and/or balancing selection have both been reported to drive the evolution of TLR genes in mammals (e.g. Ferrer-Admetlla et al. 2008; Tschirren et al. 2012) and some avian species (e.g. Alcaide & Edwards 2011).

In an analysis across 23 bird taxa we used TLR sequences from GenBank, including 10 species from New Zealand (Grueber & Jamieson 2013). We found evidence that episodic positive selection has played an important role in the evolution of most avian TLRs, consistent with their role in pathogen recognition and a hypothesis of host–pathogen coevolution (Grueber et al. 2014). Therefore, when general estimates of putatively functional genetic diversity are of interest, examining TLR gene diversity may be a promising alternative (or addition) to MHC, especially for passerines, in which the MHC is especially complex causing genotype reconstruction to be difficult and/or costly (Balakrishnan et al. 2010; Bollmer et al. 2010). The reduced genomic complexity of most TLR genes may help overcome many of these issues (Grueber et al. 2014).

Recent investigations of TLR diversity have focused on model species, but whether these genes are variable in bottlenecked populations, where genetic variation is usually

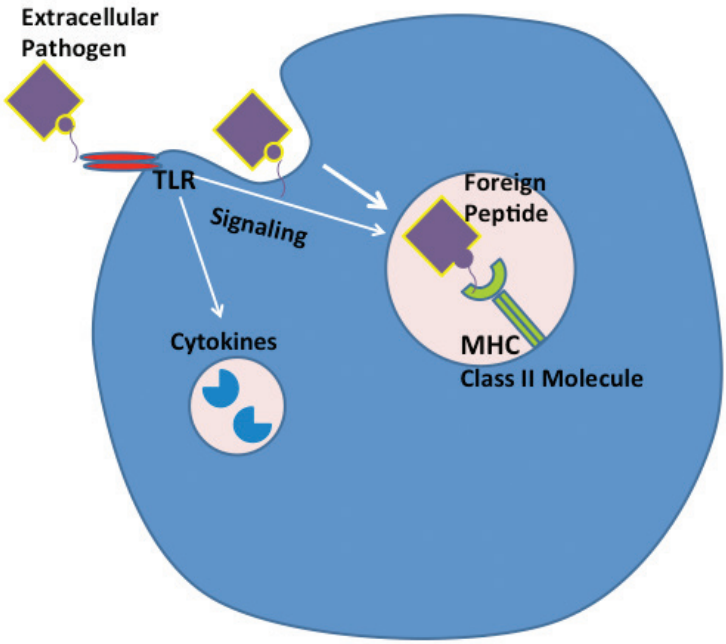

Figure 4. Toll-like receptor (TLR) genes mediate innate immune responses via recognition of pathogen-associated molecular patterns (PAMPs) and initiate a cascade of intracellular signaling to cytokines proteins, which initiate inflammation and immune responses, as well as to the acquired immune response of MHC molecules. TLRs are designed to detect extracellular pathogens (e.g. flagella of bacteria as shown in the diagram) and initiate the immune response. There are 10 TLR loci (associated with different PAMPs) known in avian species.

greatly reduced, had not been explored. We report for the first time on the level of variation at TLR loci in a bottlenecked population of Stewart Island robin (Grueber et al. 2012). Previously common and widespread, the Stewart Island robin population declined to <300 individuals in approximately 1% of its former habitat since the introduction of mammalian predators over the last four centuries (Harper 2009). A further bottleneck event occurred when, in 2000/01, 25 Stewart Island robins were reintroduced to Ulva Island sanctuary (257 ha).

We genotyped 24 robins from Ulva Island, including the 12 genetic founders, for nine TLR loci, seven of which were polymorphic (Grueber et al. 2012). We observed a total of 24 single-nucleotide polymorphisms overall, 15 of which were non-synonymous, representing up to five amino-acid variants at a locus. Levels of haplotype diversity were low (2–5 haplotypes per locus for seven loci) compared with those reported for similar-sized samples of house finch (*Carpodacus mexicanus*) and lesser kestrel (*Falco naumanni*) (respectively, 2–20 haplotypes for eight loci and 2–16 haplotypes for 10 loci; Alcaide & Edwards 2011). Nevertheless, the TLR diversity observed was sufficient to justify their further use in addressing conservation genetic questions, even in bottlenecked populations. To our knowledge, this study is the first multigene assay of TLR genetic diversity in a threatened species (Grueber et al. 2012).

During population establishment, genetic drift can be the key driver of changes in genetic diversity, but natural selection can also play a role in shaping diversity at functionally important loci. In a follow-up study on the Ulva Island robins, we used a dataset based on 722 pedigreed individuals to determine whether selection shaped genetic diversity at TLR immunity genes, over a 9-year period of population growth following establishment with 12 genetic founders (Grueber et al. 2013). We found no evidence for selection operating with respect to TLR diversity on first-year overwinter survival for alleles, loci and genotypes studied, with one exception: survival of individuals with the $TLR4_{BE}$ genotype was significantly improved, with individuals carrying the $TLR4_{BE}$ genotype less than half as likely to die prior to maturity compared with those carrying all other *TLR4* genotypes. However, comparison of observed levels of gene diversity to predictions under simulated genetic drift revealed results consistent with neutral expectations for all loci, including *TLR4*. Therefore, although selection favoured $TLR4_{BE}$ heterozygotes in this population, these effects were insufficient to outweigh genetic drift across all loci. This was the first empirical study to show that genetic drift can overwhelm natural selection in a wild population immediately following establishment (Grueber et al. 2013).

In many cases, measuring individual-level heterozygosity to understand and mitigate the role of inbreeding depression is approached by assaying levels of microsatellite diversity, and inference is extended to functional genomic regions. We have recently compared, for the first time, individual-level microsatellite-based multilocus heterozygosity (MLH) to heterozygosity at innate immunity TLR genes in 10 threatened New Zealand birds across four avian orders, species that have all been subject to population bottlenecks (Grueber et al. in press). We observed a predicted negative association between microsatellite internal relatedness (a measure of individual homozygosity) and TLR heterozygosity. This trend was consistent across all 10 species, but had a very wide error. We concluded that a signal of genome-wide heterozygosity is contained within microsatellite MLH for individuals of each of these species, but that the predictive power of this signal is poor. Furthermore, in these 10 bottlenecked species, we detected more evidence of genetic drift than balancing selection at TLR loci, with the possible exception of two species (at *TLR1LA* for hihi (*Notiomystis cinctus*) and *TLR5* for kōkako) (Grueber et al. in press). We encourage further research to be undertaken on these two species.

Future research

Our most recent research, summarised above, provides valuable data on the role of TLR diversity in disease resistance of natural populations of conservation concern. We have nine TLR sequencing results for 10 New Zealand threatened birds, which we hope will be used by researchers to study specific disease issues or problems associated with sourcing genetic diversity for reintroductions. In a collaborative study with Catherine Grueber (now at University of Sydney) and Bruce Robertson (University of Otago), we are currently examining the loss of diversity in neutral microsatellites and in MHC and TLR immunity genes in a Dunedin robin population that experienced both a prolonged bottleneck over the last 150 years and a short-term bottleneck associated with reintroduction of 16 genetic founders into a fenced sanctuary at Orokonui (307 ha). We also plan to determine whether the moderate variation in survival and reproductive success of robins in the 'predator-free' sanctuary (unpubl. data) is associated with presence/absence of immunity genes. In a further collaborative study with Sol Heber and Jim Briskie of Canterbury University, we are examining the effects of TLR and MHC diversity on survival of a reciprocal translocation of robins from two independently inbred populations in the Marlborough Sounds. We also know that John Ewen of the Zoological Institute of London will be using TLR loci to look at disease issues in hihi. Our hope is that the overall molecular ecology and pedigree fieldwork we have conducted so far will lead to better understanding of managing close inbreeding and loss of genetic diversity in small or reintroduced populations of native species.

Finally, while this review focused on inbreeding depression and loss of genetic diversity in small natural populations or in human-assisted reintroductions, we also worked on related topics that were not directly related to questions of conservation genetics (Appendices 1 and 2). It is hoped that all of the conservation issues mentioned in the review, as well as those listed in the appendices, can be either expanded or critiqued by other students and colleagues in the near future.

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Appendix 1. A summary of background studies from Jamieson's research group and collaborators on the behaviour, ecology and population dynamics of takahē and how these influenced viability in the natural population in the Murchison Mountains, Fiordland, and in the introduced populations on offshore islands. Studies are listed in chronological order of publication date.

Year	Title of publication	Journal citation
1995	New approaches towards a better understanding of the decline of Takahe (*Porphyrio mantelli*) in New Zealand.	Bunin JS, Jamieson IG. Conservation Biology 9: 100–106
1996	Responses to a model predator by New Zealand's endangered Takahe and its closest relative, the Pukeko.	Bunin JS, Jamieson IG. Conservation Biology 10: 1463–1466
1996	A cross-fostering experiment between the endangered takahe (*Porphyrio mantelli*) and its closest relative, the pukeko (*P. porphyrio*).	Bunin JS, Jamieson IG. New Zealand Journal of Ecology 20: 207–213
1997	Low reproductive success of the endangered Takahe *Porphyrio mantelli* on offshore island refuges in New Zealand.	Bunin JS, Jamieson IG, Eason D. Ibis 139: 144–151
1997	Survival and recruitment of captive-reared and wild-reared Takahe in Fiordland, New Zealand.	Maxwell JM, Jamieson IG. Conservation Biology 11: 683–691
1998	Estimating the home range and carrying capacity for takahe (*Porhyrio mantelli*) on predator-free offshore islands: implications for future management.	Ryan CJ, Jamieson IG. New Zealand Journal of Ecology 22: 17–24
2000	Increased egg infertility associated with translocating inbred takahe (*Porphyrio hochstetteri*) to island refuges in New Zealand.	Jamieson IG, Ryan CJ. Biological Conservation 94: 107–114
2000	Detecting sperm on the perivitelline membrane of incubated turkey eggs and its implications for research on fertility problems in endangered species.	Small AO, Schlusser K, Ryan CJ, Jamieson IG. Wildlife Research 27: 635–637
2003	No evidence that dietary nutrient deficiency is related to poor reproductive success of translocated takahe.	Jamieson IG. Biological Conservation 115: 165–170
2003	Immediate and long-term effects of translocations on breeding success in Takahe *Porphyrio hochstetteri*.	Jamieson IG, Wilson GC. Bird Conservation International 13: 299–306
2005	Lack of movement of stoats (*Mustela erminea*) between *Nothofagus* valley floors and alpine grasslands, with implications for the conservation of New Zealand's endangered fauna.	Smith DHV, Jamieson IG. New Zealand Journal of Ecology 29: 45–52
2012	Demography of takahe (*Porphyrio hochstetteri*) in Fiordland: environmental factors and management affect survival and breeding success.	Hegg D, Greaves G, Maxwell JM, MacKenzie DI, Jamieson IG. New Zealand Journal of Ecology 36: 75–89
2013	Use of Bayesian population viability analysis to assess multiple management decisions in the recovery program of the takahe.	Hegg D, MacKenzie DI, Jamieson IG. Oryx: The International Journal of Conservation 47: 144–152

Appendix 2. A summary of background studies from Jamieson's research group and collaborators on the behaviour, ecology and habitat selection of South Island saddlebacks and Stewart Island robins. Studies are listed in chronological order of publication date.

Year	Title of publication	Journal citation
2003	The distribution and current status of New Zealand Saddleback *Philesturnus carunculatus*.	Hooson S, Jamieson IG. Bird Conservation International 13: 79–95
2003	Breeding biology of the South Island saddleback (*Philesturnus carunculatus carunculatus* Callaeatidae).	Hooson S, Jamieson IG. Notornis 50: 191–199
2004	Variation in breeding success among reintroduced island populations of South Island Saddlebacks *Philesturnus carunculatus carunculatus*.	Hooson S, Jamieson IG. Ibis 146: 417–426
2005	Capture and handling of saddlebacks during pre-nesting does not affect timing of egg-laying or reproductive success.	Jamieson IG, Grant JL, Beaven BM. Notornis 52: 81–87
2005	Habitat selection by South Island saddlebacks and Stewart Island robins reintroduced to Ulva Island.	Steffens KE, Seddon PJ, Mathieu R, Jamieson IG. New Zealand Journal of Ecology 29: 221–229
2007	Sex determination of South Island saddlebacks (*Philesturnus carunculatus carunculatus*) using discriminant function analysis.	Taylor SS, Jamieson IG. Notornis 54: 61–64
2007	Factors affecting the survival of founding individuals in translocated New Zealand Saddlebacks *Philesturnus carunculatus*.	Taylor SS, Jamieson IG. Ibis 149: 783–791
2007	Phrase types, repertoire size and repertoire overlap in the South Island saddleback (*Philesturnus carunculatus carunculatus*).	Ludwig K, Jamieson IG. Notornis 54: 203–215
2008	Multi-scale habitat models for reintroduced bird populations: a case study of South Island saddlebacks on Motuara Island.	Michel P, Dickinson KJM, Barratt BIP, Jamieson IG. New Zealand Journal of Ecology 32: 18–33
2009	Microclimate of natural cavity nests and its implications for a threatened secondary-cavity-nesting passerine of New Zealand, the South Island Saddleback.	Rhodes B, O'Donnell C, Jamieson I. Condor 111: 462–469
2009	The roles of predation, microclimate and cavity abundance in the evolution of New Zealand's tree-cavity nesting avifauna.	Rhodes BK, O'Donnell CFJ, Jamieson IG. Notornis 56: 190–200
2010	Habitat selection in reintroduced bird populations: a case study of Stewart Island robins and South Island saddlebacks on Ulva Island.	Michel P, Dickinson KJM, Barrett BIP, Jamieson IG. New Zealand Journal of Ecology 34: 237–246
2010	Assessment of protocols and best-practice techniques learned during a translocation of South Island saddlebacks *Philesturnus carunculatus* from Ulva Island to Orokonui Ecosanctuary, New Zealand.	Masuda BM, Smith ED, Jamieson IG. Conservation Evidence 7: 69–74
2012	Rat-wise robins quickly lose fear of rats when introduced to a rat-free island.	Jamieson IG, Ludwig K. Animal Behaviour 84: 225–229
2013	Response of a reintroduced bird population to a rat reinvasion and eradication.	Masuda BM, Jamieson IG. New Zealand Journal of Ecology 37: 224–231
2014	Anti-coagulant rodenticide brodifacoum detected in dead nestlings of an insectivorous passerine.	Masuda BM, Fisher P, Jamieson IG. New Zealand Journal of Ecology 38: 110–115
2014	Differences in vocalisations, morphology and mtDNA support species status for New Zealand saddleback *Philesturnus* spp	Parker KA, Ludwig K, King TM, Brunton DH, Scofield RP, Jamieson IG. New Zealand Journal of Zoology 41: 79–94