

How does the 50/500 rule apply to MVPs?

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The 50/500 rule has been used as a guiding principle in conservation for assessing minimum viable effective population size (N_e). There is much confusion in the recent literature about how the 500 value should be applied to assess extinction risk and set priorities in conservation biology. Here, we argue that the confusion arises when the genetic basis for a short-term N_e of 50 to avoid inbreeding depression is used to justify a long-term N_e of 500 to maintain evolutionary potential. This confusion can result in misleading conclusions about how genetic arguments alone are sufficient to set minimum viable population (MVP) thresholds for assessing the extinction risk of threatened species, especially those that emphasize that MVPs should be in the thousands to maintain evolutionary potential.

The 50/500 rule and extinction risk

Early doubt and controversy about whether genetic factors had any part to play in extinction risks of threatened organisms [1–3] have now mostly disappeared [4,5]. Nevertheless, misunderstandings remain over the importance of the role of genetics in recent extinctions, and its relative contribution to the risks of threatened species [6]. One area where we believe there is still considerable misunderstanding is the role that genetics has in assessing MVP.

The 50/500 rule was proposed by Franklin [7] and became a popular guiding principle in conservation genetics for assessing MVP (see Glossary) [8]. Franklin suggested, as a rule-of-thumb, that the genetic N_e in the short term should not be <50, and in the long term should not be <500. N_e is a measure of the genetic behavior of a population relative to that of an ‘ideal’ population. It is defined as the size of an ideal population that would result in the same level of inbreeding or genetic drift as that of the population under study (Box 1). Although both 50 and 500 refer to genetic N_e , the rationale for these thresholds is based on very different genetic principles.

The short-term ‘50’ rule was based on the experience of animal breeders who observed that selection for performance and fertility could not overcome inbreeding depression when the inbreeding coefficient (F) increased by 2–3% per generation. Under the assumption that inbreeding depression might be more pronounced in wild than in domestic animals, a 1% increase in F per generation was taken as a conservative estimate [7,8]. Using the equation $\Delta F = 1/2N_e$, a 1% change in F per generation

corresponds to an N_e of 50. Therefore, an N_e of at least 50 was deemed desirable to reduce the likelihood of extinction in the short term because of harmful effects of inbreeding depression on demography [7,8].

The long-term ‘500’ rule was based on the theoretical N_e required to balance the loss of additive genetic variation per generation due to genetic drift by the creation of new genetic variation due to mutation, for a selectively neutral trait. Based on the estimated mutation rate of abdominal bristle number in *Drosophila*, and assumptions about the heritability of this trait, Franklin [7] calculated that an N_e of at least 500 was necessary to maintain a balance between drift and mutation and, therefore, over the long term, for a population to retain sufficient quantitative genetic variation to allow future adaptive change (i.e., evolutionary potential). A subsequent review of the literature revealed that the ratio of N_e to the total or census population size averaged approximately 0.10 [9], although the estimates were highly variable across species (Box 1). Therefore, in theory, an N_e of 500, or a census population size of approximately 5000 individuals, would be required to prevent the loss of quantitative genetic variation.

Of course, genetic considerations are just one of several factors that influence conservation policies and strategies, but it is generally agreed that the maintenance of genetic variation is an important factor in managing endangered species [4,5]. Nevertheless, we believe that there is still much confusion in the literature about how the 500 value, of the 50/500 rule, should be applied to assess extinction risk and prioritizations in conservation biology. In this review, we first illustrate how confusion can arise by

Glossary

Demographic stochasticity: differences in the dynamics of a population that are the effects of random events on individuals in the population.

Inbreeding coefficient: a measure of the level of inbreeding in a population, which determines the probability that an individual has two alleles at a locus that are identical by descent. It can also be used to describe the proportion of loci in an individual that are homozygous.

Minimum viable population (MVP): the minimum population size at which a population is likely to persist over some defined period of time with a given probability of extinction.

N_e/N_c ratio: the ratio of effective-to-census population size is a useful value for inferring N_e from N_c (or vice versa).

Population viability analysis (PVA): the general term for the application of models that account for multiple threats (i.e., demographic, environmental, and genetic) facing the persistence of a population to access the likelihood of the persistence of the population over a given period of time.

Quantitative genetic variation: genetic variation affecting quantitative traits, such as body size, reproduction or behavior, due to the cumulative effects at many loci.

Box 1. A brief history of the N_e/N_C ratio and its variability across species and population

Heterozygosity is lost because of genetic drift at a rate of $1/2N$ per generation in an 'ideal' (Wright–Fisher) population of N individuals; that is, one of a constant size in which the next generation is produced by drawing $2N$ genes at random from a large gamete pool to which all individuals contribute equally. This model also assumes selective neutrality so that allele frequencies change only by genetic drift. Wright [41] defined the N_e as the number of individuals that must be substituted in the formula $(1/2N)$ to describe the actual loss in heterozygosity. The assumptions of the ideal population are never met in real populations. Thus, the N_C , (i.e., the number of individuals in a study area or population) tends to be much larger than the N_e .

Frankham [8] provided the first comprehensive review of estimates of N_e in over 100 species of animals and plants. He concluded that estimates of the N_e/N_C ratio averaged approximately 0.10 in natural populations for studies in which the effects of unequal sex ratios, variance in reproductive success, and fluctuations in population size were included. More importantly, the actual N_e/N_C value in a particular population or species differs tremendously depending upon demography and life history. Frankham [8] reported that the N_e/N_C ratio varied from 0.00001 in a marine mollusk to greater than 0.30 for some low fecund species. A more recent review found a median N_e/N_C of approximately 0.15, but again, the ratio varied tremendously among species [20]. Although many low-fecundity vertebrate species have N_e/N_C ratios above 0.15, high-fecundity groups, such as marine fishes, have extremely low N_e/N_C ratios (<0.001) [20]. Therefore, the N_e/N_C ratio to use with a particular species should be estimated from values observed within similar taxa (e.g., monogamous birds, marine fishes, etc.) rather than using a single estimate derived from all taxa. In addition, the N_e/N_C ratio can vary within species; for example, the ratio has been found to increase as N_C decreases in several species [42]. Therefore, more research is required to understand changes in the N_e/N_C ratio before it is possible to infer one from the other reliably.

examining a recent debate over whether MVP size should be in the thousands based on genetic arguments alone. We then go on to discuss the role of gene flow in maintaining evolutionary potential in small, local populations and the empirical evidence regarding loss of evolutionary potential and enhanced extinction risk. We conclude by arguing that the misapplication of the 50/500 rule to MVP is partly a consequence of confusing the different timescales at which genetic and deterministic processes work, as well as confusing genetic goals with demographic goals. By reviewing some of the complexity and confusion surrounding concepts such as minimum population size, N_e , genetic viability and evolutionary potential, we attempt to provide a reappraisal of the 50/500 rule in assessing extinction risk.

Genetically derived MVPs

The use and abuse of MVPs in conservation management has had a long history of controversy [10,11]. More recently, a debate has arisen over whether MVPs should generally be in the thousands rather than the hundreds [12–17]. For example, Traill *et al.* [12] argued that a useful benchmark or threshold value for MVPs would be >5000 individuals (using an N_e of >500), based partly on the genetic theory of maintenance of quantitative genetic variation [7]. Because most conservation agencies set their target goals at a few hundred individuals (at least for many large and highly threatened vertebrates), Traill *et al.* [12] further argue that such programs '...might be managing inadvertently or implicitly for extinction – a clearly illogical or

counter-intuitive aspiration.' ([12] p. 32). Traill *et al.* [12] and Clements *et al.* [13] went on to show how the threshold value of 5000 could be utilized in conservation triage.

Flather *et al.* [14,15], among others [16], report several major analytical problems in Traill *et al.* [12]. However, the main assertion of Flather *et al.* [14,15] (that the generalization of MVP to a single threshold value is not scientifically defensible) was only applied to Traill *et al.*'s empirically derived MVP and not to the theoretically derived evolutionary MVP of Franklin [7]. This is why in their reply to the above criticisms, Brook *et al.* [17] stated this point: 'Flather *et al.* completely side-step the issue of genetic erosion in small populations, and the substantial evidence that inbreeding does indeed matter profoundly for extinction risk' and that, therefore, '...the genetic arguments alone are sufficient to embrace MVP generalisations.' ([17] p. 619).

We believe that this statement is inaccurate because it uses the genetic basis for a short-term N_e of 50 (i.e., to avoid inbreeding depression) to justify a long-term N_e of 500 (i.e., to retain genetic variation) for setting a threshold value for MVP. This could result in misleading conclusions about the use of genetics in general, and the 50/500 rule in particular, for assessing the extinction risk of threatened species. Next, we examine in more detail the genetic arguments and empirical evidence put forward in support of MVPs of 5000.

Complications in estimating of N_e and N_e/N_C ratios for evolutionary MVPs

Evolutionary MVP is defined by Traill *et al.* [12] as the MVP '...to retain evolutionary potential in perpetuity...' ([12] p. 30). This equates to the equilibrium population size where loss of quantitative genetic variation by genetic drift is offset by gains through mutation, resulting in adequate amounts of quantitative genetic variation. There has been considerable debate over exactly how large a population must be to maintain 'adequate' amounts of quantitative genetic variation (for a full discussion, see [4], p. 357), with the initial estimate putting it at an N_e of 500 [7,18], whereas others think it is closer to 5000 based on deleterious mutations and the efficacy of selection [19].

However, the currency of standard MVPs derived from simulations and population viability analysis (PVA) is not based on N_e , but on N_C , the census population size. Therefore, Traill *et al.* [12] converted the lower estimate of the drift–mutation threshold ($N_e = 500$) to its equivalent N_C using another published estimate, the median N_e/N_C ratio. Based on a relatively limited data set of the published literature at the time, the median N_e/N_C ratio across all taxa was found to be approximately 0.10 [9]. However, it might be inappropriate to apply the N_e/N_C ratio as a 'general rule' as Traill *et al.* [12] did because it simply varies so much across, and even within, species (Box 1).

Even if there is considerable uncertainty and disagreement over both the estimates of N_e at a minimum desirable drift–mutation equilibrium point and estimates for the N_e/N_C ratio, the argument that evolutionary potential will be retained in population sizes in the thousands and not hundreds is likely to be true. Nevertheless, populations in the thousands rather than in the hundreds are also

likely to have very different population dynamics to do with migration and gene flow. For example, an analysis based on molecular data shows that N_e is strongly correlated with genetic diversity (heterozygosity) only when populations are truly isolated, and genetic diversity is lost substantially over time only when populations are both small ($N_e < 100$) and isolated [20]. Unaccounted gene flow can clearly uncouple N_e estimates from processes of genetic drift, especially for local populations. Caution is needed before assuming that a focal population constitutes one single random mating unit, because violation of this basic assumption means that true N_e is underestimated or may not apply to local scales, thereby complicating inferences of genetic stochasticity and evolutionary potential based on N_e [20]. Very little gene flow into a population can maintain substantial genetic variation, even in populations with a local N_e of much less than 500 [21]. It is important to remember that the 500 value refers to the global rather than the local N_e and, therefore, it is not necessary to maintain a local N_e of 500, as long as there is some gene flow into a population. This uncertainty of scale emphasizes the importance of linking population genetic parameters with ecological and evolutionary processes [22].

However, applying caution when estimating N_e or the N_e/N_C ratio is only one of the issues in the current debate over MVP and evolutionary potential. Next, we argue that more serious problems arise when genetic parameters are not linked directly to changes in vital rates and population growth, especially when the primary aim is to estimate or inform extinction risk.

The relation between genetic viability and population viability

PVAs can take into account the effects of the 50 part of the 50/500 rule by including the accumulation of inbreeding and its negative effects on survival rates [23,24]. However, standard PVA analyses do not take into consideration how the loss of genetic variation can affect the long-term ability of a population to evolve because there is not enough information available to include mutation and its long-term effects on population viability in such models.

Traill *et al.* [12] saw the inability to evaluate the 'potential' reduced ability of small populations of conservation concern to adapt as a weakness of standard MVPs, and, therefore, devised their so-called 'evolutionary MVP', based on an N_e threshold of 500. As such, evolutionary MVPs assume that there are simple linear correlations between population size and genetic diversity, genetic diversity and population fitness, and population fitness and risk of extinction. Traill *et al.* [12] state that: 'Small populations can persist in the wild for some time, but the reproductive fitness of these, and especially the ability to adapt to change (evolutionary potential) is compromised and extirpation is likely.' ([12] p. 30).

Traill *et al.* [12] also cite the comparative study of Spielman *et al.* [25] for evidence that reduced evolutionary potential of genetically compromised populations leads to an increased risk of extinction. Spielman *et al.* [25] has been cited a total of 247 times (Web of Science), including in several related articles [17,26,27] where the authors interpret Spielman *et al.*'s results as evidential support for a

direct link between genetics and extinction risk. The premise of the study by Spielman *et al.* was the counter-argument that many researchers assert: that species are usually driven to extinction well before genetic factors have time to affect them. Spielman *et al.* predicted that if this assertion were true, then there would be little difference in genetic diversity between threatened species and taxonomically related, non-threatened species.

Spielman *et al.* [25] found that average heterozygosity was lower in threatened taxa in 131/170 comparisons, which is a significant departure from the predictions of the 'no genetic impact' hypothesis. They claimed that the differences in heterozygosity indicate lowered evolutionary potential, compromised reproductive fitness, and elevated extinction risk in the wild, and concluded that most taxa are not driven to extinction before genetic factors can affect them adversely.

The reduced heterozygosity that Spielman *et al.* detected in their comparative analysis was measured primarily at neutral loci. Indeed, Spielman *et al.* noted that the reduced heterozygosity made no direct contribution to the current threatened status of any of the taxa in their study. A quick glance at the list of threatened species used in their analysis indicates that their threat status was a result of the usual agents of decline, such as human harvesting, hunting, exploitation, habitat loss, and introduced predators. Instead, Spielman *et al.* argued that reduced genetic diversity is a 'marker' that indicates reproductive fitness is already compromised and, thus, that extinction risk is elevated, although they provide no empirical evidence that the threat status of the populations or species they analyzed would be diminished if their genetic diversity was somehow enhanced.

There are three major problems with the Spielman *et al.* [25] study and how its conclusions affect justifications for evolutionary MVPs of 5000. First, and foremost, because reduced genetic diversity is a symptom of endangerment and not normally its cause [3,4], the conclusions drawn by Spielman *et al.* clearly confound correlation with causation: that is, lower genetic diversity does not necessarily equate to elevated extinction risk. Studies of the New Zealand endemic bird, the saddleback (*Philesturnus c. carunculatus*; Box 2), illustrate that genetic diversity is a correlate of historical isolation and population size, but is a poor predictor of extinction risk and threat status per se, especially when typical deterministic drivers of population declines, such as introduced predators and habitat loss, are not taken into account [6].

Second, Spielman *et al.* [25] generate a prediction about genetic differences between extant threatened and non-threatened species that is uninformative with respect to the causes of extinctions. Indeed, some have even argued that comparative studies in general may have questionable utility when it comes to predicting extinction risks [28]. Furthermore, the reason why many species can be driven to extinction before genetic factors have time to impact is because the rate of population decline is typically far greater than that predicted on a per generational basis due to genetic factors, such as inbreeding depression. When populations are already declining (due to deterministic factors), inbreeding depression has negligible effects

Box 2. The mismatch between genetic diversity and extinction risk in South Island saddlebacks

The saddleback *Philesturnus c. carunculatus* (Figure 1a) from the South Island of New Zealand was widespread and common throughout mainland forests before the arrival and dispersal of mammalian predators across the South Island by the mid-1800s [43]. In addition to being naïve of mammals, saddlebacks were particularly vulnerable to predatory rats and mustelids because they feed, roost, and nest on or close to the ground. Consequently, saddlebacks were extirpated from the mainland by the early 1900s [43]. By 1960, the only remaining population of South Island saddlebacks was on Big South Cape Island and two small adjacent islets, off the southern coast of Stewart Island (Figure 1b). When rats were accidentally introduced to Big South Cape in 1961, 36 saddlebacks were caught and translocated to rat-free Big Island (21 birds) and Kaimohu Island (15 birds) before the Big South Cape population was extirpated. Saddlebacks prospered on Big and Kaimohu Islands and these sites became the source populations for a recovery program that eventually saw their reintroduction on 22 island sanctuaries around the South Island. The population now totals over 2000 birds and its IUCN threat status was reduced from 'Near Threatened' to 'Least Concern' [44].

DNA samples from extant populations on Big and Kaimohu islands, as well as samples taken from museum specimens from extirpated populations, indicated that historical populations living on the mainland had much higher levels of genetic and allelic diversity than did the isolated population that was on Big South Cape Island, or the contemporary reintroduced populations [45] (Figure 1c). Yet, it was the mainland population that was extirpated first, in response to the introduction of mammalian predators. By contrast, the genetically depauperate population of saddlebacks on the island of Big South Cape persisted simply because rats failed to arrive due to its isolation from the mainland. Therefore, genetic diversity was correlated to the degree of historical isolation and historic population size, but was a poor indicator of extinction risk and threat status in the face of specific agents that can drive population declines. The current low levels of genetic diversity of the recovered saddleback populations are of concern, especially at sites established well outside the historical range of the original source population and where emerging diseases are evident [46]. However, preventing reinvasion of mammalian predators should take management priority because this risk is insensitive to population N_e or levels of genetic diversity [38].

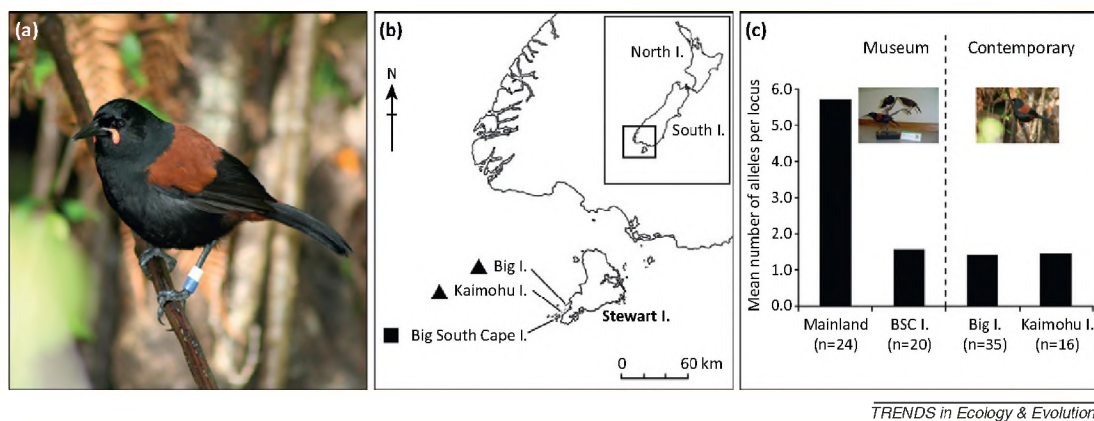


Figure 1. The decline and recovery of South Island saddlebacks *Philesturnus c. carunculatus* is predicted by its degree of isolation from introduced predators and not by genetic diversity. (a) The South Island saddleback was historically a common forest passerine belonging to the endemic wattlebird family Callaeidae of New Zealand. (b) Saddlebacks declined catastrophically after the introduction of mammalian predators to one remnant population on Big South Cape (BSC) Island, Stewart Island. After rats were accidentally introduced to BSC Island, 36 saddlebacks were caught and translocated to rat-free Kaimohu Island and Big Island in 1964. (c) Substantially higher levels of allelic diversity were found in the historic mainland population of saddlebacks than in the historic BSC Island population or in the contemporary reintroduced populations on Big and Kaimohu islands (for details, see [46]). Therefore, levels of genetic diversity are not negatively correlated with extinction risk but with the degree of historical isolation, which, in the case of saddlebacks, allowed for longer persistence on offshore islands than on the mainland. Reproduced, with permission, from R. Laws (a).

because of the slower and delayed effects of inbreeding across generations [29,30]. Therefore, estimates of reduced heterozygosity in populations of conservation concern should be linked directly to estimates of reduced fitness associated with inbreeding depression before invoking increased extinction risk due to genetic factors [23].

Third, Spielman *et al.*'s [25] comparative analysis is held up by Traill *et al.* [12] and others [17,26,27] as empirical evidence of reduced evolutionary potential of genetically compromised populations leading to an elevated risk of extinction. Yet, Spielman *et al.*'s study focuses almost exclusively on decreased heterozygosity and inbreeding depression, and not on the loss of adaptability and evolutionary potential, as the process causing genetically compromised populations to decline. As noted earlier, the effects of inbreeding depression on individual fitness and population growth can normally be incorporated in standard PVAs, such as those generated by VORTEX [31], but the potential harmful effects of loss of adaptability on

population viability cannot. Therefore, the contention that there is evidence of a clear and unambiguous link between N_e (or N_C), evolutionary potential, and extinction risk remains unsupported in the literature.

A problem of different timescales

There is little doubt that, for long-term evolutionary persistence, the N_C to ensure genetic viability is likely to be in the thousands rather than the hundreds. By contrast, the loss of genetic diversity for a population that numbers in the thousands is likely to be slow because the rate of loss of heterozygosity is proportional to $1/2N_e$. Furthermore, populations with reduced genetic variation may still be able to persist in stable or predictable environments [32]. Overall, the projected time to extinction for populations in their 1000s (but $N_e < 500$), which are potentially endangered by reduced evolutionary potential but not by other negative deterministic processes, will be long relative to the estimated times to extinction facing most contemporary populations of threatened species.

Populations numbering in the thousands, and that have reduced evolutionary potential due to low levels of genetic drift, are not the sort of populations that concern most conservation practitioners [28]. Instead, the securing of relatively small populations from extinction due to factors or processes related directly or indirectly to human activity while trying to maintain significant levels of genetic diversity is the immediate concern of conservation managers and, therefore, they tend to produce short-term recovery plans and strategies that typically deal with MVPs in their hundreds and not thousands. From a genetics perspective, although these populations might be small, many have declined recently from much larger populations and so contain considerable genetic variation that will be lost rapidly as the population approaches drift equilibrium. These are the ones that should particularly concern conservation geneticists.

Of course, numbers alone are no guarantee against extinction and so accurate prioritization of species for conservation purposes will also require knowledge of their life history, ecology, and the threats that have caused them to decline in the first place, in addition to cultural, aesthetic, taxonomic distinctiveness, and other value judgments [33,34]. Controversial prioritization schemes that rely on the 5000 individual threshold (i.e., $N_e > 5000$), such as the SAFE index [13], ignore these factors and consequently rank highly endangered species such as the kakapo (*Strigops habroptilus*), the only flightless, lek-breeding parrot in the world and sole representative of a monotypic genus, as a lower priority than more numerous but much less taxonomically distinctive species (Box 3). The kakapo example illustrates how recovery programs can set interim goals over realistic time frames and with MVP in the hundreds, while simultaneously managing genetic diversity during the

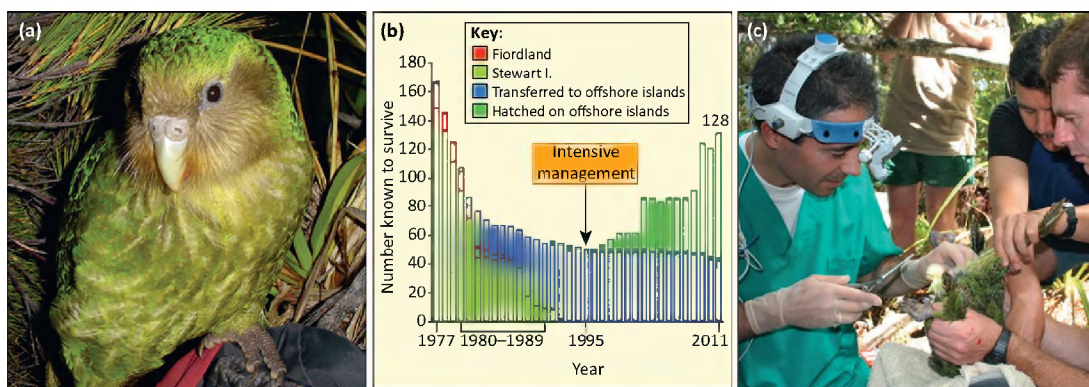
Box 3. Are endangered species such as the kakapo a lost cause?

The Australasian news media recently reported that conservation managers are wasting money trying to save 'lost-cause' species such as the hairy-nosed wombat (*Lasiornis krefftii*) of Australia or the critically endangered parrot, the kakapo (*Strigops habroptilus*) of New Zealand, for which the targeted goal of recovery is a few hundred individuals rather than several thousand [Google: 'kakapo (or wombat) not worth saving']. These reports were based on a recently published paper that advocates the adoption of a quantifiable measure of a 'species' ability to forestall extinction', the SAFE index, which could then be used to prioritize species for conservation funding [13]. The authors argue that research across a range of taxonomic groups indicates that the MVP ensuring long-term persistence (approximately 1000 years) and evolutionary potential was approximately 5000 adult individuals [12]. Therefore, they suggested that conservation funding could be prioritized according to how far the estimated number of adults of a species is below the threshold of 5000, and the likelihood and cost of elevating the species above that threshold [13].

Along with its prominence in the media, the SAFE index has attracted criticism from conservation biologists [47–49]. In the case of the kakapo, the management goal is to have an MVP of 150 female

kakapo spread across at least three island sanctuaries while maintaining current levels of genetic diversity [50]. Once 150 females are reached, intensive hands-on management (e.g., nest monitoring, captive-rearing, etc.) would cease, although the population is still predicted to grow, albeit at a slower rate [51]. Significant progress has been made towards meeting this goal (Figure 1). In addition, artificial insemination has been applied for the first time in a wild population to manage founder representation and genetic diversity during the recovery stage [52]. Managing genetic diversity at the recovery stage is essential because kakapo will be confined to islands in the foreseeable future until new technology is available to control introduced mammalian predators that are now widespread in its historical range on the mainland.

What would happen if managers considered the kakapo a 'lost cause' because the current MVP was well below the 5000 threshold of the SAFE index, and all management was stopped immediately? Modeling indicates that instead of reaching the target of 150 adult females in just 11 years, it would take three times as long to reach that goal (G. Elliott, unpublished). Worse still, the species would probably go extinct if biosecurity operations to prevent the introduction of non-native predators to offshore islands were not maintained [51].



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Figure 1. The endangered kakapo *Strigops habroptilus* illustrates how recovery goals can be set over relatively short but realistic time frames and with minimum viable populations (MVPs) in the hundreds, while managing genetic diversity. (a) The highly endangered kakapo is a large, flightless, lek-breeding parrot endemic to New Zealand and with a total population of 128 individuals, including 64 females (40 adults and 24 juveniles <6 years old). (b) When kakapo were discovered on Stewart Island during the 1970s, the population was already in sharp decline due to feral cat predation. The decline slowed during the 1980s when birds were translocated to offshore islands and the population began to recover when a suite of intensive management methods were introduced in 1995. Kakapo breed every 3 years, on average, during periodic abundant fruit crops of podocarp tree species. (c) A female kakapo is artificially inseminated with sperm collected from a wild male, the first time this technique has been used as a tool to manage genetic diversity in a wild bird population. Reproduced, with permission, from I. Jamieson (a) and B. Robertson (c). Data courtesy of New Zealand Department of Conservation (b).

crucial recovery stage [35–38]. Indeed, Bessinger *et al.* [39] have argued that choosing impossible or unrealistic genetic goals might have weakened the initial popularity of using N_e as a concept in management. Others have proposed including N_e along with other factors, such as amount of habitat loss and chance of catastrophe, when devising criteria for listing endangered species [40]. What is of primary importance is that factors affecting N_e estimates are given full consideration without holding managers to specific numeric goals [39].

Concluding remarks

We believe that inbreeding depression is fully accounted for in standard PVAs and for estimates of MVP size resulting in the '50' component of the 50/500 rule. We agree that the need to maintain long-term genetic diversity for evolutionary potential is an important component of conservation programs. However, we see MVPs of N_e of approximately 500, or its much larger N_C equivalent, as a long-term aspirational goal for maintaining healthy and genetically robust populations, and not a threshold trigger that predicts extinction risk [12] or facilitates triage decisions to allocate resources in conservation [13]. We advocate management strategies that emphasize the maintenance of genetic diversity during the recovery stage of conservation programs [38]. We prefer this approach over one that focuses almost exclusively on the requirement to reach a minimum recovery size of thousands of individuals to maintain evolutionary potential in perpetuity, and that claim to do otherwise is a waste of time.

The 50/500 rule was developed over 30 years ago. Despite all the advances in molecular and population genetics since then, these simplistic point estimates of N_e thresholds for avoiding the harmful consequences of inbreeding depression (50) and retention of quantitative genetic variation for future adaptations (500), remain unchanged. We believe that the 50/500 rule still has a useful place in conservation biology, as long as it is used as a guiding principle to indicate when genetic concerns are likely to have an important role in the short- and long-term viability of populations.

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50/500 rule and minimum viable populations: response to Jamieson and Allendorf

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There are many assertions in Jamieson and Allendorf's recent review in *TREE* [1] (JA2012) that are either incorrect, or contradict current knowledge, the material they cite (especially [2–4]), or their own publications [4–6]. Their review also includes contradiction and misrepresentation of published work. Given space and citation constraints, here we only address some key issues, and reference mainly reviews.

Erroneous attribution

JA2012 claimed that Spielman *et al.* [7] 'noted that the reduced heterozygosity made no direct contribution to the current threatened status of any of the taxa in their study.' In fact, we wrote 'We were unable to determine whether genetic factors have contributed to the current threatened status of the taxa in our study.' [7].

Misrepresentations of the relation between genetic factors and extinction

JA2012 also stated 'the conclusions drawn by Spielman *et al.* clearly confound correlation with causation: that is, lower genetic diversity does not necessarily equate to elevated extinction risk.' However, we stated 'Each of the essential links between reduced genetic diversity and subsequent extinction risk has been verified' [7], and followed with multiple, independent lines of supporting evidence. Similarly, Jamieson *et al.* [5] wrote 'Empirical studies have confirmed direct links between inbreeding and/or loss of genetic variation and population viability...'

JA2012 stated that 'estimates of reduced heterozygosity in populations of conservation concern should be linked directly to estimates of reduced fitness associated with inbreeding depression before invoking increased extinction risk due to genetic factors'. There is already extensive theoretical and empirical evidence for such links in many circumstances, as documented in [7] and elsewhere [2,3,5].

Predicted links

Theory predicts causal links between neutral genetic diversity, genetically effective population size (N_e), and

inbreeding coefficient (F) in closed random mating populations [2–4,7] (Equation 1):

$$\frac{H_t}{H_0} = \left(1 - \frac{1}{2N_e}\right)^t = 1 - F \quad [1]$$

where H_0 is initial heterozygosity and H_t is heterozygosity after t generations. Because F is causally related to inbreeding depression for reproductive fitness [2–4,7], this links heterozygosity and inbreeding to fitness. Census population size (N_c) is also linked when N_e and N_c are positively correlated [8].

Empirical tests support these predictions

First, 'inbreeding depression is a universal phenomenon' (Allendorf and Luikart [4]). Second, N_e and N_c are correlated with genetic diversity for allozymes, microsatellites, and major histocompatibility complex (MHC) variation [3,8]. Third, genetic diversity and fitness are positively correlated across populations [2,3,7,8]. Fourth, inbreeding increased extinction risk in experiments where other causes were excluded or controlled [2,3,7]. Fifth, inbreeding substantially reduces median times to extinction in wild populations, based on population viability analyses for well-studied species [2,3,7]. Sixth, inbreeding increased extinction risk in natural habitats in the three species where this was seriously examined [2,3,7]. Seventh, loss of alleles at self-incompatibility loci (S-alleles) reduced fitness in self-incompatible plants [2–4]. Indeed, the Illinois lakeside daisy was effectively extinct due to low S-allele diversity, but recovered following outcrossing [2,3]. Eighth, reduced genetic diversity at the sex locus in Hymenoptera elevated predicted extinction risk [3,4].

JA2012 stated 'they [7] provide no empirical evidence that the threat status of the populations or species they analyzed would be diminished if their genetic diversity was somehow enhanced.' We [7] wrote 'small natural populations of a topminnow fish, a greater prairie chicken, and a Swedish adder all have declined in numbers, in part because of inbreeding, and recovered after outbreeding.' Indeed, outcrossing inbred populations to unrelated conspecific populations with the same karyotype and environmental adaptations usually enhances fitness, as documented by Darwin, plant and

animal breeders, and studies of laboratory and wild populations [2,3,9].

JA2012 incorrectly concluded ‘the contention that there is evidence of a clear and unambiguous link between N_e (or N_c), evolutionary potential, and extinction risk remains unsupported in the literature.’ Theory predicts such links [2,3] and they are supported empirically [10]. We [7] stated ‘reduced genetic diversity has been shown to reduce times to extinction under changing environments’, and similarly Allendorf and Luikart [4] said ‘loss of genetic variation due to inbreeding made these lines [10] less able to adapt to continuing environmental change.’ Similarly, Jamieson *et al.* [5] concluded ‘inbreeding in small populations almost invariably leads to loss of genetic variation, and such populations are less responsive to selection pressure.’ Furthermore, species that evolved heavy-metal tolerance when repopulating polluted areas in Wales had pre-existing genetic variation for tolerance, whereas those that failed did not [2,3].

JA2012 claimed that we [7] incorrectly conflated arguments about evolutionary potential and inbreeding depression. Yet Equation 1 predicts these are related in random mating populations, because inbreeding depression is causally related to F [2–4]. This theory applies here, because derivation of the $N_e = 500$ rule assumed neutral quantitative genetic variation [3,4]. Furthermore, the near-neutral theory of molecular evolution and genomic evidence indicates that populations of $N_e = 500$ are not immune to inbreeding depression, because fixed genetic loads still occur in populations with $N_e > 500$ [3]. For example, numbers of duplications and mobile elements, and larger introns (all usually deleterious) are negatively related to N_e , over a range of approximately 10^4 – 10^8 [11]. Furthermore, the ratio of synonymous to nonsynonymous base changes (the latter resulting in altered amino acids) in humans ($N_e \approx 10\,000$) and murids ($N_e \approx 1\,000\,000$) are 0.23 and 0.13, respectively, indicating differences in fixed genetic loads, even at $N_e > 500$ [12].

JA2012 commented: ‘A quick glance at the list of threatened species used [7]... indicates that their threat status was a result of the usual [human associated] agents of decline...’. However, Jamieson and Lacy [6] stated ‘We now recognize that genetic factors do not normally work in isolation, but instead often interact with factors that cause

populations to decline in the first place...’, and [7] and others [2–4] made comparable statements.

Contradiction

The many assertions made by JA2012 (detailed above) about the lack of evidence for causal connections between genetic factors, population demography, and extinction risk contradict their statement that ‘Early doubt and controversy about whether genetic factors had any part to play in extinction risks of threatened organisms... have now mostly disappeared...’, because establishing the causal links between genetic factors and extinction risk were critical to resolving the controversy.

We are preparing an extended critique of JA2012 for another journal.

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A school of red herring: reply to Frankham *et al.*

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In their Letter in *TREE* [1], Frankham *et al.* criticize several aspects of our review of how to apply the 50/500 rule to minimum viable populations (MVPs) [2]. Unfortunately, they do not address our primary conclusion, that

the 500 in the 50/500 rule should not be used as a threshold to make triage decisions to allocate conservation resources. Neither do they address our assertion that converting 500 to a threshold of 5000 individuals using an N_e/N_c (effective over census population size) ratio of 10% is not appropriate because of the great variability in this ratio observed

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among species [2]. Rather, their primary point is that we assert incorrectly that there is a 'lack of evidence for causal connections between genetic factors, demography, and extinction risk' [1]. Their entire Letter provides detailed evidence for this point. Unfortunately, their reply largely overlooks our point that the justifications for the 50 and the 500 in the 50/500 rule are fundamentally different.

We are in complete agreement with them that there is an overwhelming amount of empirical justification for the importance of having an N_e of at least 50 to avoid increasing risk of extinction because of inbreeding depression. This evidence, however, does not justify a long-term N_e of at least 500. As we point out, the long-term '500' rule was based on the theoretical N_e required to balance the loss of additive genetic variation per generation due to genetic drift by the creation of new genetic variation due to mutation, for a selectively neutral trait. This rule cannot be justified by evidence that inbreeding depression has demographic effects and can increase the probability of extinction in small populations. In their reply [1], Frankham *et al.* argue that molecular evidence for fixed genetic loads in populations with $N_e > 500$ indicate that such populations 'are not immune to inbreeding depression'. This is a red herring. The observation that genetic drift and inbreeding will affect even very large populations does not provide evidence that inbreeding depression will reduce the long-term viability of populations with an $N_e < 500$. Frankham *et al.* present further evidence for increased fixed genetic load in populations with $N_e > 10\,000$. A logical extension of their reasoning is that long-term N_e values should be $> 10\,000$.

We agree with Frankham *et al.* on how inbreeding depression and reduced genetic diversity affect individual fitness and population viability. Nevertheless, our review falls at a different end of the spectrum on how these genetic factors should be taken into consideration in the management of threatened populations. The response of Frankham *et al.* focuses on how genetic factors can play a major role in increasing the risk of extinction. Our review of the role of the 50/500 rule in conservation management focused on the dynamics and consequences of small populations, especially when those populations are stable or increasing slowly (i.e., at the recovery stage of a threatened species when the main agents of decline are under control

or have been eliminated). We then addressed the question of what is the minimum size a recovering population needs to reach to avoid the harmful effects of inbreeding depression in the short term and to retain sufficient genetic variation to allow adaptive change over the long term. In this context, the 50/500 rule is most relevant to providing guidelines for managing population recovery, rather than preventing extinction *per se*.

We also warned against using the '500' aspect of the 50/500 rule as a management requirement, where setting recovery goals with an $N_e < 500$, or its much larger N_C of 5000, is '...managing inadvertently or implicitly for extinction...' [3]. We also strongly disagree that recovery programs with short-term recovery goals in the hundreds, rather than thousands, should be penalized with respect to prioritization of conservation funds [3,4]. We believe that Frankham *et al.* place excessive weight on inbreeding depression in very large populations and the potential risk of extinction associated with reduced selection response in future environments.

In conclusion, we fully agree with Frankham *et al.* that it is important to take genetic factors into account when managing species of conservation concern. It is for this reason that we conclude, as we did in our original review, that the 50/500 rule has a useful place in conservation management as a guiding principle when genetic concerns are likely to affect the short- and long-term viability of populations.

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The founder space race: a response to Waters *et al.*

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We welcome the attempt by Waters *et al.* [1], in the spirit of the journal that they are publishing in, to address scales not typically dealt with by ecology (too long) or evolution (too short). The basic scenario that they address [i.e., the expansion of the range of a taxon (species,

population, or genotype) into empty niche space], is a nonequilibrium situation in which many ecological processes occur. However, the timescales they refer to range from very short (a few generations) to very long (thousands to millions of generations). They suggest that a 'founder takes all' principle might explain reduced genetic diversity in recently colonised areas. We are concerned that their use of the term 'density-dependent

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