



Perspective

Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses

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ARTICLE INFO

Article history:

Received 15 May 2013

Received in revised form 16 December 2013

Accepted 28 December 2013

Keywords:

Effective population size

Evolutionary potential

Fragmentation

Gene flow

Inbreeding depression

Minimum viable population size

ABSTRACT

Conservation managers typically need to make prompt decisions based on limited information and resources. Consequently, generalisations have essential roles in guiding interventions. Here, we (i) critique information on some widely accepted generalisations and variables affecting them, (ii) assess how adequately genetic factors are currently incorporated into population viability analysis (PVA) models used to estimate minimum viable population sizes, and (iii) relate the above to population size thresholds of the IUCN Red List criteria for threatened species that were derived from genetic considerations. Evidence accumulated since 1980 shows that genetically effective population size (N_e) = 50 is inadequate for preventing inbreeding depression over five generations in the wild, with $N_e \geq 100$ being required to limit loss in total fitness to $\leq 10\%$. Further, even $N_e = 500$ is too low for retaining evolutionary potential for fitness in perpetuity; a better approximation is $N_e \geq 1000$. Extrapolation from census population size (N) to N_e depends on knowing the ratio of N_e/N , yet this information is unavailable for most wild populations. Ratio averages (~ 0.1 – 0.2) from meta-analyses are sufficient, provided adjustments are made for dissimilar life histories. Most PVA-based risk assessments ignore or inadequately model genetic factors. PVA should routinely include realistic inbreeding depression, and genetic impacts on evolutionary potential should be incorporated where appropriate. Genetic generalisations used in conservation, the treatment of genetics in PVAs, and sections of the IUCN Red List criteria derived from genetic considerations, all require revision to be more effective conservation tools.

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Abbreviations: d , dominance; MVP, minimum viable population size; N_e , effective population size; N , census population size; PVA, population viability analysis; q_e , equilibrium frequency; s , selection coefficient; V_A , additive genetic variation; V_m , mutational variation for a quantitative character; u , mutation rate.

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1. Introduction

Conservation biology is a crisis discipline requiring urgent management for threatened species often with inadequate information (Soulé, 1985). As most species have inadequate information on which to base effective intervention decisions, conservation action is frequently opportunistic, seeking compromise under competing demands, and/or politically expediency (Pressey and Bottrill, 2008). For example, parcels of land offered for sale require that decisions to purchase for conservation must be made promptly (McDonald-Madden et al., 2008). Given limited resources and sparse information for most threatened species, scientific generalisations are often required. Some authors have criticised these (Flather et al., 2011), but the alternative is usually unscientific conservation decisions made at the political and bureaucratic levels, especially in poorer countries and for non-charismatic species (Brook et al., 2011).

The International Union for Conservation of Nature and Natural Resources (IUCN) recognises the need to conserve biodiversity at three level: genetic diversity, species and ecosystems (McNeely et al., 1990), with genetic issues being involved in all three (Frankham et al., 2010). We concentrate on the first two. Species are usually driven to extinction by a combination of systematic human-associated threats (habitat loss, over-exploitation, introduced species, pollution and climate change) and stochastic events associated with small population size (demographic, ecological and genetic stochasticity, and catastrophes) (Shaffer, 1981), typically interacting in a synergistic feedback (Brook et al., 2008) termed the 'extinction vortex' (Gilpin and Soulé, 1986). In this *Perspective* we focus on controversial aspects of genetic stochasticity (see Glossary in Appendix A1), primarily encompassing inbreeding depression, and reduced evolutionary potential (Frankham et al., 2010; Jamieson and Allendorf, 2012).

Inbreeding and loss of genetic diversity are unavoidable in small, closed, sexually reproducing populations, and accumulate in a ratchet-like manner over generations for diploid random-mating populations, as follows (Wright, 1969):

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

where H_t is heterozygosity at generation t (for neutral variation), H_0 initial heterozygosity, N_e genetically effective population size and F the inbreeding coefficient (with generation zero defined as having $F = 0$). In naturally outbreeding species, this typically results in inbreeding depression (unless they have already experienced it) and reduced ability to evolve (Frankham et al., 2010).

To work, generalizations depend on different taxa responding similarly, or at least groups of them doing so. While ecologists typically emphasise species distinctiveness (e.g. Flather et al., 2011), evolutionary and conservation geneticists usually focus on the similarity of evolutionary processes across species with similar breeding systems. For example, across most major taxa: (i) inbreeding has consistently deleterious effects on fitness in wild outbreeding diploid and polyploid species (Crnokrak and Roff,

1999), (ii) population mean genetic diversity, mean fitness and population size are positively correlated (Frankham, 2012), (iii) heritabilities (genetic variation as a proportion of phenotypic variation) are lower for fitness than for quantitative traits peripherally related to fitness (Mousseau and Roff, 1987; Falconer and Mackay, 1996), (iv) non-additive genetic variation is greater for fitness than peripheral traits (Frankham et al., 2010), and (v) mutation rates for quantitative characters are relatively similar (Houle et al., 1996). Consequently, generalisations are often justifiable for genetic issues in conservation biology (see also Appendix A2).

Our focus is on three genetic issues relating to generalisations. First, an effective population size of at least 50 (Franklin, 1980; Soulé, 1980) has been long recommended as a 'rule' for avoiding inbreeding depression in the short term. Second, $N_e = 500$ has been considered sufficient to retain evolutionary potential in perpetuity (Franklin, 1980; Lande and Barrowclough, 1987). These two issues had important roles in the development and implementation of the IUCN Red List categorisation system for threatened species (Mace et al., 2008), especially criterion C that relates to population size (Appendix A3). Third, minimum viable population sizes (MVP; Shaffer, 1981) provide estimates of the sizes required for species to persist with high probability in the long-term. Given that over 30 years have elapsed since the classic $N_e = 50$ and $N_e = 500$ recommendations were proposed, and their tenacity in conservation management circles, we now ask whether current evidence supports them, and how they might be modified. Jamieson and Allendorf (2012) reviewed these issues, but we reach different conclusions to them on several important issues.

We critique and make recommendations (Table 1) on (i) the $N_e = 50$ and 500 rules, (ii) how best to translate N_e into census population size (N), (iii) the genetic consequences of fragmented populations, and (iv) the treatment of genetic issues in PVA and their effect on estimation of MVPs. Finally, we (v) evaluate the implications of these for the IUCN Red List categorisation system.

2. Population size required to avoid inbreeding depression in the short term: $N_e = 50$?

Soulé (1980) and Franklin (1980) proposed $N_e = 50$ as sufficient to prevent inbreeding depression in naturally outbreeding diploid species in the short term, and most authors, including Jamieson and Allendorf (2012) still endorse this value. However, specifying the duration as 'short term' is too vague, because it can mean different things in different disciplines. Since Soulé and Franklin had ~5 generations in mind (from discussions with RF), we recommend that 5 generations be used (because genetic effects scale to generations).

Franklin (1980) and Soulé (1980) based their $N_e = 50$ on the opinion of animal breeders, plus limited data from domestic and laboratory animals. However, inbreeding depression is generally greater in stressful, wild environments than in benign, captive ones (Fox and Reed, 2010), and domestic animals might have different susceptibility to inbreeding depression compared to wild populations because they have been subjected to artificial selection,

Table 1
Topics addressed in this perspective.

Topic	Current recommendation	Revised recommendation
1. Avoid inbreeding depression	$N_e = 50$	$N_e \geq 100$
2. Maintain evolutionary potential	$N_e = 500$	$N_e \geq 1000$
3. Extrapolating from N_e to N	$N_e/N = 0.10\text{--}0.14$	Use different N_e/N according to life-history of species, but current default is 0.1–0.2
4. Fragmented populations and connectivity	Evaluate on case basis	Evaluate, but distinguish current and historical gene flow
5. Genetic factors in PVA	Often none	Routinely include inbreeding depression
(i) Inbreeding depression	Lethal equivalents = 3.14 on juvenile mortality, if included	Routinely apply realistic levels (~12 lethal equivalents on total fitness)
(ii) Evolutionary potential	Ignored	Include in long-term and environmental-change contexts
6. MVPs	Persistence probability inconsistent	Apply common standard and specify (suggest 99%)
	Duration inconsistent	Standardise and specify in generations (suggest 40)
	Important factors often ignored	Routinely include all systematic and stochastic factors (including genetic)
7. IUCN Red List Criterion C	Population size	
Critically Endangered	<250	<500
Endangered	<2500	<5000
Vulnerable	<10,000	<20,000

adaptation to captive environments and frequent population-size bottlenecks (Frankham, 2009; Mattila et al., 2012).

Subsequent to 1980, inbreeding depression has been reported in many laboratory populations with $N_e \geq 50$ (Latter and Mulley, 1995; Bryant et al., 1999; Reed and Bryant, 2000; Woodworth et al., 2002). Even housefly populations with $N_e \sim 90$ for 5 generations exhibited inbreeding depression for fitness (Bryant et al., 1999). Current information predicts an average decline in total fitness of 26% in the wild due to 5 generations at $N_e = 50$ (cumulative $F \sim 5\%$) (see computations in Appendix A4) based on 12 diploid lethal equivalents in wild environments from the meta-analysis in O'Grady et al. (2006), while the 16 lethal equivalents value from Grueber et al. (2010) yields a decline of 33%. Further, Newman and Pilson (1997) found that when F increased by 5%, population extinctions rose from 25% to 69% in *Clarkia pulchella* plants. These values represent intolerably high inbreeding depression in the wild.

Since most studies report linear relationships between F and fitness (Frankham et al., 2010), $F > 0$ will usually cause adverse fitness effects in natural outbreeding species. For conservation management, we suggest specifying that a 10% decline in fitness is tolerable (following similar logic to the captive breeding objective of retaining 90% of genetic diversity for 100 years; Frankham et al., 2010). Theory predicts that $N_e = 142$ is required for a population to retain 90% of current fitness after inbreeding of $F = 5\%$, based on 12 diploid lethal equivalents. Given limited data on lethal equivalents and the potential for modest purging (see Appendix A5), we suggest that the N_e be raised to at least 100 and re-evaluated as more data become available.

2.1. Overview and recommendations

In summary, we recommend that the generalisation for avoiding inbreeding depression in the short term should (i) specify an explicit duration of 5 generations; (ii) recognise that any $F > 0$ will usually have adverse fitness effects; (iii) be defined as an N_e that leads to <10% cumulative decline in total fitness in the wild, and (iv) stipulate that minimum N_e be increased to 100.

3. Population size required to maintain evolutionary potential in perpetuity: $N_e = 500$?

Franklin (1980) argued that additive quantitative genetic variation (V_A), rather than single-locus variation determined the ability to evolve. He concluded that $N_e = 500$ was sufficient to retain

evolutionary potential in perpetuity, based on the equilibrium between adding genetic variation by mutation (V_m), and losing V_A ($2N_e$) per generation by random genetic drift for a quantitative trait that is either unaffected by selection or subject to stabilising selection (typical of peripheral characters). He then solved for N_e using empirical data on V_m and the heritability of the trait, as detailed in Frankham et al. (2010). Lande and Barrowclough (1987) reached a similar conclusion based on a model of mutation, drift and stabilising selection on a peripheral character. However, more recent alternative quantitative genetic theory has yielded N_e values ≥ 1000 (Appendix A6). Jamieson and Allendorf (2012) queried the association of N_e , evolutionary potential and extinction, despite extensive theory and empirical evidence (field, laboratory and simulation data) supporting it (see Appendix A7), but they generally endorsed the $N_e = 500$ value. While Franklin (1980) considered only evolutionary potential and quantitative genetic variation in the context of $N_e = 500$ it cannot be entirely divorced from concerns about inbreeding depression (Frankham et al., 2013; Appendix A8).

Subsequent considerations of N_e to retain evolutionary potential raised issues of the fitness of new mutations and the appropriate value of V_m (see Appendix A9), and led us to ask whether evolutionary potential should be determined for peripheral or fitness traits.

3.1. Re-evaluating evolutionary potential

By definition, the evolutionary adaptation of populations by natural selection involves changes in reproduction and/or survival rates (reproduction fitness), even if the changes also affect other characters. Fitness traits have fundamentally different features from peripheral characters, especially in the predominant forms of natural selection experienced, asymmetry of mutation effects, non-additive genetic variation and the maintenance of genetic variation (Table 2). As there is no agreed value of N_e required to retain evolutionary potential for fitness in perpetuity, we compile several lines of evidence to provide a preliminary solution.

Genetic variation for fitness in a closed, random-mating population is maintained by the balance between mutation (adding it), drift (removing it) and selection (either removing or retaining variation). Much of the genetic variation is maintained by a balance between addition of deleterious mutations, and their removal by selection and drift (Charlesworth and Hughes, 2000; Rodríguez-Ramilo et al., 2004; but see Charlesworth et al., 2007), resulting in equilibria with low frequencies of deleterious alleles that are susceptible to loss by drift (see below).

Table 2

Differences in characteristics of reproductive fitness traits versus peripheral quantitative characters (after Frankham, 2009).

Characteristic	Quantitative character	
	Fitness	Peripheral
Inbreeding depression and heterosis	Strong	Weak
Asymmetry of selection response	Strong	~Symmetrical
Heritabilities	~10–20%	~30–70%
Non-additive genetic variation	Present	Little or none
Effect of mutation on mean	Reduces	Minimal change
Genotype × environment interactions	Stronger	Weaker
Natural selection	Directional	Stabilising

We divide alleles at fitness-related loci into five categories (near recessive lethals, mildly deleterious, ones experiencing balancing selection, beneficial, and neutral in the current environment), and determine what N_e is required to maintain approximately the same genetic diversity as expected in an infinite population. We concentrate on the first three, because the Franklin (1980) and other theory described above applies to the small proportion that is neutral in the current environment, while the few categories that are beneficial in the environment to which the population is adapted (Perfeito et al., 2013) are rapidly fixed or lost by drift, so few are polymorphic at any given time. Of these categories, it is the mildly deleterious alleles that are likely to be of predominant importance in adapting to changed environments, as they are most frequent, and because lethals and other highly deleterious alleles are usually unconditionally deleterious (Lindsley and Zimm, 1992). Alleles that are deleterious in one environment can contribute to adaptation because they are often beneficial in others: genotype × environment interactions (Frankham et al., 2010), including home-site advantage (Hereford, 2009) and adverse fitness effects experience by captive-adapted populations in wild habitats (Frankham, 2008).

3.1.1. Lethals

Species' genomes typically contain rare lethal alleles at many loci. For example, ~5000 loci in *Drosophila* potentially have lethal alleles whose heterozygotes have an average reduction in viability of ~2.5% (ds) per generation compared to wild-type homozygotes, and new lethal mutations arise at a rate (u) of $\sim 3 \times 10^{-6}$ locus⁻¹ generation⁻¹ (Simmons and Crow, 1977). The balance between deleterious mutations and selection against heterozygotes for such alleles in an infinite diploid population results in an equilibrium frequency (q_e) of:

$$q_e \sim u/ds \quad (2)$$

where d is the dominance of the allele (proportionate deviation of heterozygote fitness from the mean of the homozygotes) and s the selection coefficient against the deleterious homozygote. Consequently, lethals ($s = 1$) have low equilibrium frequencies at individual loci ($\sim 1.2 \times 10^{-5}$) in infinite populations. Their equilibrium frequencies depend on N_e up to at least $N_e = 1000$ (Nei, 1968; Hedrick, 2002; not independent of N_e as stated by Lande and Barrowclough, 1987). Average lethal frequencies in experimental *Drosophila melanogaster* populations maintained with $N_e = 500$ for 51–67 generations were substantially less than that in a recently caught sample of the wild base population (Fig. A1), consistent with the above theory.

3.1.2. Mildly deleterious alleles

Deleterious partial recessives (at many loci) with average heterozygous disadvantages ~ 1 –5% on viability (Houle et al., 1996) are predicted to have equilibrium frequencies of 2×10^{-4} to 10^{-3} in infinite populations (assuming $u \sim 10^{-5}$ and the viability effects

are representative of total-fitness effects). Genetic diversity for partially recessive alleles ($d < 0.5$) at single loci begin to asymptote by $N_e = 1000$ when $s \geq 0.01$, based on theoretical analyses of inbreeding depression (a function of the heterozygosity for such alleles; Falconer and Mackay, 1996), and fixed deleterious allele frequencies (Bataillon and Kirkpatrick, 2000).

Visible mutations (due to mildly deleterious alleles) in *Drosophila* populations maintained with N_e of 25, 50, 100, 250 and 500 for 51–67 generations showed, on average, progressively increasing frequencies of chromosomes containing unique visible mutations as N_e was raised, but a recently wild-caught sample of the base population had a 37% higher frequency than the $N_e = 500$ treatment (Fig. A2), consistent with the above theory.

3.1.3. Alleles subject to balancing selection

Few loci appear to experience balancing selection and have intermediate allele frequencies (Charlesworth and Hughes, 2000; Bubb et al., 2006). In an infinite population, the equilibrium frequency for a locus subject to heterozygote advantage (relative fitnesses for genotypes A_1A_1 , A_1A_2 and A_2A_2 of $1 - s$, 1 and $1 - t$, respectively) is:

$$q_e = \frac{s}{s + t} \quad (3)$$

Such loci are less affected by drift than neutral loci when equilibrium frequencies are 0.2–0.8, but are more affected outside this range (Robertson 1962; Hedrick, 2012). Robertson concluded for fitness loci subject to heterozygote advantage in nature that “the effective population size must be of the order of 1000 before the genetic variation found within it approaches the value for very large populations . . .” Similarly, for frequency-dependent selection, Roff (1998) argued on the basis of multi-locus simulations “that the heritability increases with population size, typically approaching its maximal value at a population size of 5000, when the mutation rate is 10^{-4} , and double this when the mutation rate is 10^{-5} .”

Maintenance of genetic variation by selection that varies temporally or spatially only occurs under quite restricted conditions, based on theoretical models (Prout, 2000). Further, empirical studies indicate that such selection is implicated in maintaining variation at a low proportion of loci (Hedrick, 2006). We found no relevant theory relating genetic variation to N_e for such selection.

3.2. Overview and recommendations

Several independent lines of theoretical and empirical evidence indicate that at least $N_e = 1000$ is required to maintain initial evolutionary potential in perpetuity. We recommend the minimum N_e required to maintain evolutionary potential equal to that of large wild populations in perpetuity be revised to (i) specify maintenance of genetic diversity for reproductive fitness, (ii) be derived from theoretical models involving mutation, selection and drift, plus empirical data, and (iii) specify N_e of at least 1000 in naturally outbreeding species. Populations with $N_e < 1000$ are not doomed to extinction in the short to medium term, but their ability to evolve to cope with environmental change will erode with time and this will reduce their long-term viability.

4. Extrapolating from effective (N_e) to census (N) population sizes

N_e discussed above need to be converted into mean (adult) census population sizes (N) per generation for conservation managers. Information on the N_e/N ratio is required for this conversion, but comprehensive estimates that encompass all relevant variables are only available for ~100 species (Frankham, 1995; Palstra and Ruzzante, 2008; Palstra and Fraser, 2012). Accordingly, most

conversions have been based on average N_e/N (0.10–0.14; Frankham, 1995; Palstra and Ruzzante, 2008). Palstra and Fraser (2012) reported a median ratio of 0.12, but favoured one of 0.23 from cases where appropriate N_e and N were strictly paired.

Jamieson and Allendorf (2012) stated that “the actual N_e/N value in a particular population or species differs tremendously depending upon demography and life history.” Below we consider this issue and whether extrapolating from N_e to N should be avoided. Following Mace and Lande (1991), we consider it imperative that population size guidelines be provided for conservationists working with species without N_e/N estimates.

Differences in N_e/N estimates are largely due to use of comprehensive and non-comprehensive estimates (Frankham, 1995; Leroy et al., 2013), sampling variation (Frankham, 1995; Palstra and Fraser, 2012), methodological variation (Appendix A10), use of different measures of N (adults, breeding pairs, and adults plus juveniles combined), current N not necessarily reflecting long-term N (Frankham, 1995), pairing N_e with diverse (often inappropriate) N (Palstra and Fraser, 2012), and variation in life-history traits (Waples et al., 2013). Care must also be taken to use N consistently, as both mean N (most frequent) and harmonic mean N are used (Frankham et al., 2010). However, there are constraints on this variation. Frankham (1996) concluded that “the overall relationship between heterozygosity and $\log N$ [correlation 0.81 across species] would not be possible if N_e/N ratios varied widely among different species and taxa.” Further, correlations of genetic diversity- $\log N_e$, and genetic diversity- $\log N$, are similar within species and across species (Frankham, 2012), and there is little statistical support for differences between major taxa (Frankham, 1995). Methodological variation for comprehensive N_e/N estimates appears minor (Appendix A10). Additionally, putative population-density effects (Palstra and Ruzzante, 2008; Luikart et al., 2010) were not confirmed by Palstra and Fraser (2012).

Palstra and Ruzzante (2008) reported N_e/N was much lower ($\sim 2 \times 10^{-5}$) in highly fecund exploited fish compared to other species (as predicted by theory: Waples, 2002; Hedrick, 2005), but suggested that this might either be a methodological artefact and/or a consequences of human exploitation. However, given that both exploited (fish, oysters, and shrimp) and unexploited (*Drosophila* and brown algae, *Fucus serratus*) species with high fecundity also have low N_e/N (Frankham, 1995, 2012), the role of exploitation is questionable. Further, methods other than the temporal one used in their study also yielded low N_e/N in *Drosophila* and water hyacinth (*Eichhornia paniculata*) (Frankham, 1995). Consequently, low ratios for highly fecund species could be realistic, but more information is required.

Since the meta-analysis by Palstra and Ruzzante (2008), the ongoing use of a single mean value of N_e/N for all species is unsound. Waples et al. (2013) reported that up to half the variance in single generation N_e/N was explained by age at maturity and adult lifespan, opening the possibility of providing more precise extrapolations from N_e to N than possible previously. This must be combined with information on fluctuations in population sizes across generations to yield comprehensive N_e/N estimates.

4.1. Overview and recommendation

Conversion from N_e into N must both involve comprehensive estimates of N_e and account for different N_e/N for species with diverse life histories. Mean N_e/N is ~ 0.1 – 0.2 .

5. Fragmented populations and connectivity

So far, we have considered only single, approximately random-mating closed populations. However, free-ranging wild species

usually have fragmented distributions, with population structures varying from effectively single random-mating populations (fragmented spatially, but connected by gene flow), through partially connected fragments and meta-populations, to completely isolated subpopulations; each circumstance has different genetic consequences in relation to genotype frequencies, genetic differentiation, inbreeding, fitness, and extinction risks (Table A1; Frankham et al., 2010).

Population fragmentation of species is recognised as a threatening process in the IUCN Red List Categorisation System (IUCN, 2013). However, Jamieson and Allendorf (2012) noted that unaccounted gene flow can reverse adverse genetic effects of small population size. Alternatively, connectivity might be weaker than generally recognised in species of conservation concern due to ongoing habitat loss, declining population sizes, declines of many plant pollinators (Brosi and Briggs, 2013), expansion of roads, fences and other human-constructed barriers to gene flow (Crooks and Sanjayan, 2006), increasing impacts of invasive species (Gilbert and Levine, 2013), and lower fitness of immigrants than residents (Hanski et al., 2000). How serious a genetic threat is fragmentation?

While human impacts increase connectivity for some species (especially invasives), most empirical evidence indicates limited connectivity among subpopulations of many once-contiguous species (see Table A1 where references are provided). First, >25% of species have populations that differ genetically (F_{ST} [inbreeding and drift due to population isolation] >0.2) (see Appendix A11). Second, populations within species typically exhibit correlations between genetic diversity and population size. Third, population mean fitness is typically correlated with population size. Fourth, species often show isolation by distance. Fifth, F_{ST} correlates negatively with dispersal ability. Recent research also suggests that marine organisms disperse less than previously assumed (Cowen and Sponaugle, 2009; Jones et al., 2009). Tropical trees could be an exception where fragmentation increases gene flow via pollen, but this might only occur over a restricted geographic range (White et al., 2002; Dick et al., 2008; Hamrick, 2010). Conversely, for 15/21 tropical forest tree species, low-density (generally fragmented) plants showed lower multilocus outcrossing rates compared to high-density (usually undisturbed forest) plants (Dick et al., 2008), calling into question whether overall gene flow is generally higher in these fragmented tropical tree species.

We conclude that population fragmentation is a serious genetic threat to the persistence of populations of many species.

5.1. Recommendations

We recommend that genetic connectivity continue to be assessed among populations within species to inform species management and advise that current and historical connectivity be distinguished. For previously connected populations that now have limited gene flow and evidence of genetic erosion, we recommend augmentation of gene flow be considered, provided the risk of outbreeding depression is low (Frankham et al., 2011).

6. How well is genetics incorporated into PVAs and MVPs?

Minimum viable population (MVP) sizes required for long-term population persistence are commonly determined using population viability analysis (PVA) simulation models (Traill et al., 2007). Ideally these models should include all systematic and stochastic variables potentially affecting population viability. But how much is genetics considered in most PVAs?

There is now extensive evidence that genetic factors influence extinction risk (Frankham, 2005; Frankham et al., 2010; Allendorf

et al., 2013). First, inbreeding depression substantially reduces median times to extinction in PVAs for real species in natural environments (Brook et al., 2002; O'Grady et al., 2006). Second, empirical field studies of the Glanville fritillary butterfly (*Melitaea cinxia*) and of two plant species revealed that inbreeding reduces population viability in wild populations (Newman and Pilson, 1997; Saccheri et al., 1998; Vilas et al., 2006). Third, most species are not driven to extinction before genetic factors can influence them (Spielman et al., 2004). Fourth, reduced genetic diversity for fitness compromises the ability of populations to adapt to environmental change (Appendix A6). Fifth, mutation accumulation might reduce long-term population viability, especially in asexual and selfing species (Frankham et al., 2010).

6.1. Inbreeding depression in PVAs

Jamieson and Allendorf (2012) concluded: “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.” However, “most published PVAs have not included genetics” (Allendorf and Ryman, 2002), and even in the last decade many still have not (Traill et al., 2007; Zeigler et al., 2013). Further, most PVAs that include inbreeding depression apply only 3.14 diploid lethal equivalents to juvenile survival (the default setting in the widely-used *Vortex* PVA software) (Traill et al., 2007). This underestimates the total impact of inbreeding (Allendorf et al., 2013), because the true value averages ~12 for total fitness (O'Grady et al., 2006). In addition, N_e/N in PVAs is typically higher (~0.30) than observed in unmanaged wild populations (mean 0.1–0.2), because most software default settings, including those in *Vortex*, assume Poisson distributions of family sizes (Frankham et al., 2010 p. 506), whereas species usually exhibit much larger variance (Frankham et al., 2010 p. 246), and thus lower N_e/N .

6.2. Evolutionary potential in PVAs

As long-term persistence of populations in changing environments typically requires the ability to evolve, this capacity should be included in long-term PVAs. While PVAs have not included evolutionary potential (Jamieson and Allendorf, 2012), there are few impediments to doing so. A practical and empirically tested equation exists for predicting long-term genetic adaptation (Appendix equation A2). Further, several studies have used individual-based stochastic computer-simulation models to investigate how adaptive evolutionary changes affect population persistence of fish (Dunlop et al., 2007; Reed et al., 2011; Kuparinen and Hutchings, 2012; Piou and Prévost, 2012).

6.3. Minimum viable population size

Minimum viable population size refers to the number of individuals required for a high probability of population persistence over the long term (Shaffer, 1981; Soulé, 1987). However, MVPs have been determined for few species, so average values or statistical models are all that can be used for the remainder.

MVPs estimates have used a variety of different probabilities of persistence and durations in years, or generations (99% for 1000 years: Shaffer, 1981; 95% for several centuries: Soulé, 1987; 99% for 40 generations: Traill et al., 2007). To be biologically comparable, duration should be in generations, because extinction risk scales better to generations than years (O'Grady et al., 2008). We suggest the standardised 99% probability of persistence for 40 generations used in the Traill et al. (2007) meta-analysis be routinely reported, even if other scales are also considered.

Based on the data in the Traill et al. (2007) meta-analysis, the MVP for 99% persistence for 40 generations for a typical outbreeding

species is of the order of several thousand (N). Whether major taxa differ in MVPs is unclear (Frankham et al., 2010 p. 518), but they could be higher in species with higher fecundities (see Section 4).

6.4. Recommendations

We recommend that (i) inbreeding depression be routinely included in PVA models for all sexually reproducing diploid or polyploid species with normal chromosomal segregation, (ii) inbreeding depression in PVAs be routinely applied to total fitness (i.e. survival and fertility across all age/stage classes), (iii) realistic inbreeding depression be applied for outbreeding species (~12 total diploid lethal equivalents for wild environments), but lower values for populations subjected to bottlenecks that have already purged highly deleterious alleles, (iv) fewer lethal equivalents be applied for species/populations with selfing or mixed-mating systems, (v) evolutionary potential be included in PVA models of long-term environmental change, or for estimates of indefinite persistence of species, and (vi) MVPs be reported against a standard 99% probability of persistence for 40 generations using PVAs that include all relevant systematic and stochastic variables.

7. Relationships between $N_e = 100$, $N_e = 1000$, MVP and IUCN Red List criteria

MVPs identify populations that are relatively safe from all threats. If available and based on adequate data, a species-specific PVA is the most comprehensive guide to the likely persistence for a species, or population. If this is not available, or a PVA yields results of doubtful validity, or a species falls into the Data Deficient category of the IUCN Red List, then $N_e \leq 100$ indicates that it likely faces serious genetic threats after 5 or more generations, whereas $N_e \geq 1000$ indicates little or no genetic threats to its long-term persistence: this usually translates into census population sizes (N) similar to MVPs.

As IUCN Red List population size thresholds under Criterion C derive from genetically effective population sizes (Appendix A3), and two explicitly from N_e of 50 and 500 theories, it is logical to double them to reflect the revisions we recommend above. Mace and Lande (1991) assumed $N_e/N = 0.2$ to translate effective population sizes into number of mature adults, but this may need reconsideration, as described in Section 4 above. The incorporation of genetic risks in the IUCN Red List categorisation system appear illogical and would likely benefit from revision (Appendix A12). We expect that a doubling of the above thresholds will increase the number of species falling into each of the threatened categories, but by how much needs to be determined by empirical evaluation. The results of assessments on 28 taxa (Appendix A13) showed that one third of the species that could potentially change did move.

PVA can be used to assess risk of extinction for categorisation under the IUCN Red List Criterion E, but most current values are underestimates, as described in Section 6 above.

Many will have concerns that recommended targets for effective population sizes are unattainable for most threatened species. Although increasing N might be impractical in many wild populations, N_e can also be augmented by increasing the N_e/N ratio, or re-establishing gene flow among isolated sub-populations of a species (Frankham et al., 2010). Substantial increases in N_e can usually be made if totally isolated populations of a species that were previously connected by gene flow are re-connected using artificial gene flow, or habitat corridors (Frankham et al., 2010). In some conservation circumstances, there are no practical remedies to avoid genetic deterioration and the population or species needs to be categorised as fragile with risk minimised by non-genetic

means, as done in the case of the Wollemi pine (*Wollemia nobilis*) (Frankham et al., 2010 p. 399).

8. Conclusions

Based on our review of recent theoretical and empirical evidence, we conclude that the oft-cited $N_e = 50$ rule for avoiding (minimising) inbreeding depression in the short-term (5 generations), and $N_e = 500$ proposed for maintaining evolutionary potential in perpetuity, need to be at least doubled, as do the genetically derived IUCN Red List population-size thresholds for Criterion C. Further, population viability analyses need to incorporate more realistic genetic risks by routinely including inbreeding depression on total fitness applied at levels relevant to the intended environment (wild or captive populations). For PVAs that consider long-term scenarios the effects of evolutionary potential should be modelled (and this is feasible).

Acknowledgments

We thank G. Cooke, M. Eldridge, C. Fenster, I. Franklin, P. Hedrick, W. Hill, A. Hoffmann, C. Lees, G. Mace, D. Spielman, M. Soulé, Y. Willi and five anonymous reviewers for comments on the manuscript. C.J.A.B. and B.W.B. are supported by Australian Research Council Future Fellowships.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2013.12.036>.

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