Appendix A. Supplementary material

A1. Glossary

(revised from material in Frankham et al., 2010)

Balancing selection: Selection that maintains genetic variation in a population, encompassing heterozygote advantage (overdominance), frequency-dependent selection (rare advantage), and particular forms of selection that vary over space, or time.

Directional selection: Selection in which the most extreme high- (or low-) ranked individuals on some traits from a population are most successful as parents of the next generation.

Dominance (*d*): Deviation of heterozygote mean from the mean of homozygotes at a locus.

Effective population size (N_e): The number of individuals that would result in the same loss of genetic diversity, inbreeding, or genetic drift if they behaved in the manner of an idealized population.

Environmental stochasticity: Effect of natural fluctuations in environmental conditions, such as rainfall, food supply, competitors, winter temperatures, etc., that affect a species and can drive a small population to extinction.

Evolutionary potential: The ability of a population to evolve to cope with environmental changes. Often simplistically equated with genetic diversity (especially for quantitative characters such as fitness), but it is also influenced by the selection differential, heritability of the trait and N_e as well.

Extinction vortex: Describes the likely adverse interaction between human impacts, inbreeding, and demographic fluctuation that result in a reinforcing feedback loop and spiral downwards in population size towards extinction.

F: Inbreeding coefficient. Probability that two alleles at a gene locus in an individual are identical by descent.

Fitness: Reproductive fitness, the number of fertile offspring contributed by an individual that survive to reproductive age (lifetime reproductive success).

Frequency-dependent selection: A form of natural selection where the relative fitnesses of genotypes vary with their frequencies. When rare genotypes are favoured, such selection maintains genetic variation and is a form of balancing selection.

***F_{ST}*:** Proportion of the total inbreeding in a population due to differentiation among sub-populations. Has a continuous scale from 0 to 1.

Gene flow: Movement of alleles between populations via migrants or gametes.

Genetic diversity: The extent of genetic variation in a population, or species, or across a group of species; for example heterozygosity, allelic diversity, or heritability.

Genetic drift: Changes in the genetic composition of a population due to random sampling in finite populations. Also referred to as random genetic drift.

Genetic erosion: Inbreeding depression and loss of genetic diversity in small populations.

Genetic stochasticity: Genetic consequences of small populations, including inbreeding, loss of genetic diversity due to genetic drift and chance fixation of deleterious mutations that reduce fitness and may drive a population or species towards extinction (often in combination with other factors).

Heritability: Proportion of the variation for a quantitative character due to (additive) genetic causes.

Heterozygosity: Proportion of heterozygous individuals (ones with two different alleles at a gene locus) in a population.

Heterozygote advantage: A form of selection where the heterozygote has a higher reproductive fitness than the homozygotes. Also referred to as overdominance.

Idealized population: A conceptual random-mating population with equal numbers of hermaphrodite individuals breeding in each generation, and Poisson variation in family sizes (mean = variance). Used as a standard against which other populations are equated when defining genetically effective population sizes.

Inbreeding: The production of offspring from mating of individuals related by descent e.g. self-fertilization, brother × sister, cousin matings, etc.

Inbreeding coefficient (*F*): The probability that two alleles at a locus in an individual are identical by descent. Used to measure the extent of inbreeding.

Inbreeding depression: Reduction in mean for a quantitative trait due to inbreeding, especially manifest in reproductive fitness traits.

Lethal: Inconsistent with survival, as in a recessive lethal allele that results in death when homozygous.

Lethal equivalents (*B*): A measure for comparing the extent of inbreeding depression in different populations. A group of detrimental alleles that would cause death if homozygous, e.g., one lethal allele, two alleles each with a 50% probability of causing death, etc. typically estimated from the slope of the regression of $\log_e$ (survival) on the inbreeding coefficient *F*.

Linkage: Location of different loci on the same chromosome that can result in non-random assortment of alleles into gametes.

Linkage disequilibrium: Non-random association of alleles at different loci, usually due to co-location on a chromosome.

Metapopulation: A spatially distributed group of partially isolated populations of the same species that undergo local extinctions and recolonisations.

Minimum viable population size (MVP): The minimum size of a population that is viable in the 'long term', meaning a probability of persistence of say 99% for 40 generations, or 90% for 100 years. Often determined via population viability analysis.

'Mutational meltdown': Decline in reproductive rate and consequent downward spiral in population size towards extinction due to chance fixation (drift) of new mildly deleterious mutations in small populations.

Mutation-selection balance: Equilibrium between the spontaneous occurrence of deleterious mutations and natural selection removing them, resulting in low frequencies of deleterious alleles in populations.

MVP: See minimum viable population size.

Outbred: An individual whose parents are unrelated.

Outbreeding: Not inbreeding. Approximately random mating.

Outbreeding depression: A reduction in reproductive fitness sometimes observed due to crossing of two populations (or sub-species, or species) caused, for instance, by some combination of disruption of local adaptation, fixed chromosomal differences, and long isolation.

Outcrossing: Crossing a population to another that is not closely related.

Partial dominance: The condition where the heterozygote has a phenotype closer to one homozygote than the other e.g. heterozygotes for many deleterious alleles are nearly, but not completely normal.

Population viability analysis (PVA): A systems modelling approach for predicting the fate of a population (including risk of extinction) due to the combined effects of all systematic and stochastic threats faced by a population. Typically population size, means and standard deviation of birth and death rates, density feedbacks, plus risks and severity of catastrophes, inbreeding depression, etc., are input into a software package and many replicates projected over multiple generations using stochastic computer simulation. Used as a management and research tool in conservation biology.

Purging: Reducing the frequency of deleterious alleles (frequently partial recessives) by natural selection, especially that associated with populations that have suffered size bottlenecks and/or inbreeding.

***Q_{ST}*:** Proportion of genetic variation for a quantitative trait (V_G) that is among populations, computed as V_G among populations divided by (V_G among + $2V_A$ within populations). It is the quantitative trait analogue of F_{ST}.

Quantitative character: Typically a trait with a continuous distribution influenced by genetic and environmental variation e.g. fecundity, survival, height and weight. Contrast with discrete Mendelian or particulate traits.

Quantitative genetic variation: Genetic variation affecting a quantitative character (V_G). Presumed to be due to the cumulative effects of variation at many quantitative trait loci. Also referred to as polygenic variation.

Random genetic drift: see Genetic drift.

Random mating: A pattern of mating where the chances of two genotypes or phenotypes breeding is determined by their frequencies in the population.

Reproductive fitness: See fitness.

Selection differential (S_d): A measure of the intensity of selection on a quantitative character: difference in mean between the (selected) parents and the mean of the total population from which they derived.

Stabilising selection: Selection favouring phenotypic intermediates for a quantitative character.

A2. Generalisations for genetic issues in conservation biology

To apply generalisations (rules of thumb) as is done in many human endeavours, it is not necessary for there to be no variation or exceptions, only for variation to be modest and for it to work in most cases. Typically, means are applied in developing and applying rules of thumb (as we have done), as this is the best expectation of predicted impact.

Given limited resources and inadequate information to estimate extinction risk for many species, it is important to have guidelines for minimum viable population sizes to ensure a reasonable probability of population persistence and to minimize genetic problems in species. This latter issue, first seriously raised in 1980, is still an important concern; Zeigler et al (2013) stated "Lacking this (PVA), there is a strong need for viable and comprehensive methods for determining quantitative, science-based recovery criteria for endangered species with minimal data availability." The IUCN Red List categorisation system thresholds (IUCN 2012) depend heavily upon generalisations for both genetic and other effects (see A3 below).

There are several grounds for using genetic generalisations, as done by Franklin (1980 and Soulé (1980) and those who have endorsed their 50/500 rules and used them in developing the IUCN Red List categorisation system (IUCN 2012). Here we explicitly recognise the use of generalisations and present evidence to justify them. First, theory from population and quantitative genetics that is widely applied in conservation genetics is generic - i.e., it applies equally to all diploid, sexually reproducing, outbreeding species with normal chromosome segregation (Crow and Kimura, 1970; Falconer and Mackay, 1996). Similarly, theory for inbreeders, tetraploids, or haplo-diploids applies generically. For example, generic theory applies to genetic drift, mutation-selection equilibrium, inbreeding depression and evolutionary potential (Frankham et al., 2010). Second, population sizes across populations and species are measured on an equivalent scale as N_e by equating them to an idealised population with the same genetic impacts (Falconer and Mackay, 1996). Third, input parameters such as mutation rates and heritabilities are

typically similar across populations and species for similar characters (see below). Fourth, the equations usually have good predictive power and are generally better than many of those developed in ecology - e.g., the correlation between N_e and microsatellite genetic variation among closed populations of diverse species explained 53% of the variation (r^2), and those between N and allozyme variability explained 49% and 66% of the variation among species (Frankham 2012). This compares to meta-analyses in ecology and evolution with r^2 of around 2.5–5.4% being typical (Møller and Jennions, 2002). Further, the equation predicting evolutionary adaptation over multiple generations predicts observed changes well across invertebrate, vertebrate and plant species (Weber, 2004). Empirically, evolutionary genetics principles established with *Drosophila* and other laboratory species apply to humans, maize, golden lion tamarins, giant pandas and other species with similar breeding systems.

The conditions where inbreeding depression (ID) is found or not are well understood on the basis of theoretical models and empirical observations (see Falconer and Mackay, 1996; Frankham et al., 2010). As shown below, inbreeding in naturally outbreeding diploid species has overwhelmingly deleterious effects on reproductive fitness (unless they have already experienced substantial ID). In brief, ID occurs when deleterious recessive alleles are made homozygous, and/or loci experiencing balancing selection (especially heterozygous advantage) are made homozygous (the former being more important than the latter). Thus, the occurrence of ID requires sexual reproduction and diploidy, or polyploidy (no ID for asexual or haploid species). From genetic models in random mating sexually reproducing diploid species (Falconer and Mackay, 1996 pp.249-250) the extent of inbreeding depression is expected to be:

$$\mathrm{ID} = \sum_{i=1}^{n} 2p_i q_i d_i F \qquad \text{(A1)}$$

where p and q are allele frequencies (with $2pq$ the initial heterozygosity and $2pqF$ the reduction in heterozygosity due to inbreeding) at loci affecting a quantitative character, d is the dominance deviation (deviation of heterozygote mean from the average of the two homozygotes), F the inbreeding coefficient and n the number of polymorphic loci affecting the trait.

Thus, ID requires, or has the following characteristics:

1. A load of deleterious alleles (typically coming from mutation-selection balance), or polymorphic loci exhibiting heterozygote advantage or other forms of balancing selection.
2. Directional dominance across loci. This is generated by directional selection, as found for reproductive fitness (reproduction and survival), not by stabilising selection as is presumed to be experienced by most other quantitative characters (e.g., there is little dominance for loci affecting bristle number in *Drosophila* and little or no ID). Empirical observations show greater ID for fitness than for non-fitness traits (DeRose and Roff, 1999).
3. Inbreeding ($F > 0$)
4. ID is greater with higher numbers of polymorphic loci where homozygosity has deleterious effects on fitness, as for total fitness, compared to individual

fitness components. Empirical data show that ID is typically higher for total fitness than its components (Frankham et al., 2010)

5. In populations with a history of inbreeding (e.g., selfing plants), there is less polymorphism and heterozygosity is < 2*pq*, so ID should be reduced, other things being equal (empirical observations show generally less ID in selfing than outcrossing species: Husband and Schemske, 1996)
6. In haplo-diploid species (Hymenoptera and various other invertebrate groups), there is no ID in haploid males, and selection against deleterious recessives is highly effective in males, so little ID is observed in females (as observed empirically: Henter, 2003; Kureck et al., 2012).
7. ID is usually greater in stressful than benign environments (empirical observation: Frankham et al., 2010).
8. ID might be greater in diploids than polyploids, but the issue is not fully resolved (Frankham et al., 2010).
9. Populations with a long history of small population sizes often already exhibit substantial ID, but are less susceptible to further inbreeding depression, as many loci are homozygous (e.g. Mattilla et al., 2012).
10. While populations within species, and species often differ in ID, tests typically do not reveal differences among major taxa (among mammalian orders: Ralls et al., 1988; among birds, mammals, poikilotherms, and plants: Crnokrak and Roff 1999; for total fitness among invertebrates, vertebrates and plants; R. Frankham, unpublished results). ID does differ between gymnosperm and angiosperm plants (Husband and Schemske, 1996), but this comparison is complicated by different selfing rates and frequencies of polyploidy (Frankham et al., 2010).

Overall inbreeding almost always has deleterious effects on most fitness characters (especially on total fitness) in naturally outbreeding diploid and polyploid species (albeit due to different mixes of fitness components) (Darwin, 1876; Wright 1977; Charlesworth and Charlesworth, 1987; Falconer and Mackay, 1996; Lynch and Walsh, 1998), unless they are already suffering from it from previous inbreeding (due to fixed deleterious alleles). In fact, Lacy (1997) concluded that "no species of mammal has been shown to be unaffected by inbreeding," given that studies that reported no statistical evidence of inbreeding depression had sample sizes or levels of inbreeding that were too low to have provided statistical power adequate to detect the expected fitness depression. Kalinowski and Hedrick (1999) also concluded that many experiments designed to detect inbreeding depression (especially for captive endangered species) had low statistical power.

In some studies, ID depends on the rate of inbreeding (Reed et al., 2003; Pedersen et al., 2005), but not in others (Cornelius and Dudley, 1974; Kristensen et al., 2011). Reed et al. (2003) found no difference in extinction rates from inbreeding with N_e = 10 versus N_e = 20, the comparisons of most relevance to the issues considered in this *Perspective*. The effects of different environments, selection intensities and inbreeding by environment interactions (Reed et al., 2012) can be minimised by considering separately populations in benign captive conditions from those in wild environments (as we have done).

All naturally outbreeding sexual diploid and polyploid species examined have a load of rare nearly recessive deleterious mutations (unless they are already highly inbred) that contribute to inbreeding depression when made homozygous, including loci causing genetic disorders in humans, domestic and laboratory animals and

plants and wildlife (Lewontin, 1974; Frankham et al., 2010). This genetic load depends on mutation-selection equilibrium for deleterious mutations (predicted by equations that are generic across outbreeding diploid species). Most mutations have deleterious effects on fitness for species well adapted to their environments and measured in that environment (see Vale et al., 2012; Perfeito et al., 2013), and mutational accumulation decreases mean fitness (data from various species; Frankham, 2009). Further, mutational variances are similar across species, but differ for fitness versus peripheral traits (Houle et al., 1996).

Most quantitative traits in most naturally outbreeding species have the ability to evolve in response to selection (Lewontin, 1974; Falconer and Mackay, 1996; Frankham 2009). Even where one trait does not exhibit quantitative genetic variation, others typically do (Frankham, 2009). Evolutionary potential depends on the heritability of the character, on the selection applied and on the effective population size (Weber, 2004). Heritabilities for similar characters (fitness versus peripheral) in natural outbreeders are relatively similar across species (Mousseau and Roff, 1987; Falconer and Mackay, 1996) and fitness traits typically exhibit asymmetrical response to selection across a broad array of species (more in the direction of reduced fitness: Frankham 1990).

Loss of pre-existing genetic diversity over generations is unavoidable in all closed sexually reproducing populations and species, and occurs for diploids at a rate per generation for neutral variability inversely dependent on the effective population size, as given by equation 1 in the main text. Empirical data indicate similar patterns of loss of genetic diversity across species for populations with similar N_e for near-neutral genetic markers (e.g. allozymes and microsatellites) and relatively similar patterns for loci subject to selection (reviewed by Frankham, 2012).

Consequently, generalisations are often justifiable for genetic issues such as inbreeding depression and loss of evolutionary potential in conservation biology.

A3. Relationship between N_e, 50/500, PVAs and IUCN Red List criteria for threatened species

The current version (and other recent versions) of the IUCN Red List criteria for identifying threatened species (IUCN, 2012) represents a major advance over the early (pre-1994) versions in terms of being scientifically and quantitatively based, objective, transparent and consistent. “The IUCN based these rules on Mace and Lande (1991)” (Mace et al., 2008) who defined quantitative targets for persistence for specified durations (in years and generations) (as would typically be determined using PVAs) for three categories of threatened species, Critically Endangered (CR), Endangered (EN) and Vulnerable (VU). The close connection to genetic issues is evident in the specification of population size thresholds as effective population sizes (a parameter unique to genetic issues), that were then translated into mature adults (potentially breeding adult census sizes, our N) using $N_e/N = 0.2$.

The N_e thresholds for the CR, EN and VU categories were < 50, < 500 (both as discussed in this paper) and < 2000, respectively. These had to occur in

combination with fragmentation, population decline, or a population catastrophe to trigger the threatened categorisation, and there were alternative means to reach the categorisations, but these had to result in similar characteristics to the above. Subsequent revisions removed the N_e thresholds, but retained the N values that were derived from them (Mace et al., 2008). The structure was also modified such that any one of five thresholds for Criteria A-E triggered a threatened categorisation, with Criterion C being adult population size and continued decline and Criterion D being adult population size in populations with stable sizes. Under C, declining adult population sizes of < 250, < 2500 and < 10000 trigger categorisations of CR, EN and VU, respectively (as derived from N_e < 50, < 500 and < 2000 in Mace and Lande, 1991). Under D, < 50 mature adults leads to a species being categorised as CR, < 250 as EN (presumably derived from N_e < 50) and < 1000 as VU. Further, in the final revised version of the IUCN Red List criteria, Mace et al., (2008) cite Soulé (1980) and state "it is recommended that minimum effective population sizes of at least 50 be maintained." They also say "… the population size measures are defined very specifically, adjusting the observed value to reflect the number of mature individuals after accounting for the effects of age, sex, breeding structure, and degree of population-size fluctuation." This approximates to the comprehensive effective population size (Frankham 1995). Given these explicit links, it is consequently logical to revise the current population size targets if the original underpinnings are revised, as we have recommended in the main text.

A4. Computing expected decline in total fitness in the wild due to inbreeding depression with different lethal equivalents

Following the classic Ralls et al., (1988) paper and using 12 diploid lethal equivalents ($2B$), as estimated in the O'Grady et al. (2006) meta-analysis for the impacts of inbreeding on total fitness in the wild, and with an inbreeding coefficient of 0.05, we estimate the mean reduction in relative fitness of a population in the wild due to inbreeding (δ) as:

$$\delta = 1 - e^{-FB} = 1 - e^{-0.05 \times 6} = 0.26$$

(1 – δ is the multiplier on vital rates, or overall fitness).

If we instead assume 16 diploid lethal equivalents, as per Greuber et al. (2010), δ is expected to be:

$$\delta = 1 - e^{-FB} = 1 - e^{-0.05 \times 8} = 0.33.$$

These estimates assume that inbreeding depression is the same with different rates of inbreeding, but it will usually be somewhat less with slower inbreeding (including larger N_e) because natural selection has more opportunity to remove (purge) deleterious recessive alleles that are the main cause of inbreeding depression (Reed et al., 2003) (see A2). There is no general, acknowledged correction for this, but empirical data on purging often indicate only modest effects (reviewed by Frankham et al., 2010: A5 below). Consequently, over 5 generations the above δ values should only be moderately overestimated.

A5. Purging

Purging is the removal by natural selection of deleterious (mainly partially recessive) alleles contributing to inbreeding depression. It is not effective in removing the component due to loci showing heterozygote advantage.

While the results of purging are variable, the conditions where it will be effective versus ineffectively are reasonably well understood (see Frankham et al., 2010 pp. 299-303). In brief, the efficacy of purging depends on:

- genetic load (amount and whether due to deleterious alleles, or heterozygote advantage loci),
- variation in inbreeding within the population,
- effective population sizes, and
- intensity of natural selection.

Large values of each of these are associated with high rates of purging. However, simulation studies and empirical experiments show that in small populations with sizes typical in conservation management, purging is often weak, because the effectiveness of selection diminishes as population size is reduced and variation in inbreeding is typically low in random-mating populations (see Hedrick, 1994; Frankham et al., 2001; Reed et al., 2003). For example, Boakes et al. (2007) concluded that “the mitigating effects of purging were found, on average, to be small (median < 1% decrease in inbreeding depression) and hence can be considered, overall, as having a negligible effect”, based on studies of 119 zoo populations of 88 species. Ballou (1997) reached a similar conclusion for zoo animals, as did Byers and Waller (1999) for plants, based on a review and meta-analysis. Further, Reed et al. (2003) reported inbreeding depression (extinctions) with N_e = 10 and N_e = 20 (and the two did not differ statistically, but did differ from full-sib mating) and ID has been reported in populations with up to N_e = 90 in houseflies, as documented in the main text.

Thus, in practical terms purging often has limited effects in random-mating diploid and polyploid populations of conservation concern and does not eliminate inbreeding depression (as often only deleterious alleles of large effect are purged, and deleterious mutations continue to be added each generation).

A6. Recent quantitative genetic models relevant to maintenance of evolutionary potential in perpetuity

Several recent theoretical quantitative genetic studies have concluded that $N_e \geq 1000$ is required to retain evolutionary potential in perpetuity. First, cumulative evolutionary change over *t* generations due to directional selection of constant intensity on a quantitative character ($\sum E_t$) depends on N_e, V_A, selection differential (S_d: difference in trait mean of parents and all adults in their generation), V_m, phenotypic variance (V_P) and *t*, and is predicted as (Weber and Diggins, 1990):

$$\sum E_t \sim 2\, N_e (V_A/V_P) S_d \{1 - \left[\left(1 - \frac{1}{2N_e}\right)\right]^t + \left(\frac{V_m}{V_A}\right) \sum_{i=1}^{t} 1 + \left(1 - \frac{1}{2N_e}\right)^i\} \quad \text{(A2)}$$

Empirical evidence supports this equation's predictions (Falconer and Mackay 1996; Weber 2004). From this equation Weber and Diggins (1990) concluded that "populations of 10 000 would be effectively infinite in the time span of fifty generations, and populations of 1000 nearly so.**"** Second, Willi et al. (2006) concluded that "effective population sizes in the low thousands may be sufficient to maintain adequate adaptive potential in most cases," based on quantitative genetics considerations. Third, Keightley and Hill (1987) found that the relationship between genetic variation and N_e approaches an asymptote at 10^5 for a quantitative genetic model with linkage (Willi et al., 2006). Consequently, recent quantitative genetic models indicate that $N_e = 500$ is inadequate for maintaining evolutionary potential in perpetuity and instead at least double that value is supported by the evidence.

A7. Links between N_e, evolutionary potential, population growth and extinction risk

Theory predicts that evolutionary potential is related to N_e (Robertson, 1960; Falconer and Mackay, 1996; see equation A2 above), yet Jamieson and Allendorf (2012) stated that "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."

On the contrary, links between reduced ability to evolve to cope with environmental change and extinction have been predicted by several theoretical studies (Lynch and Lande, 1993; Bürger and Lynch, 1995; Gromulkiewicz and Holt, 1995; Lande and Shannon, 1996; Orr and Unckless, 2008; Chevin et al., 2010). Further, the connection between N_e (or associated *F*) and extinction risk have been verified quantitatively in several experiments (Frankham et al., 1999, 2010, p. 238; England et al., 2003).

It is important to recognise that modest inbreeding and loss of genetic diversity often do not cause population growth to become negative and thus lead to rapid extinctions (Frankham et al., 2010 pp. 291-293). If a population initially has a

4% growth rate, a 50% decline due to inbreeding still leaves it with a 2% growth rate so that it can recover from a bottleneck, but more slowly than could the original population. These two populations might also reach similar carrying capacities, despite different reproductive fitness, due to density feedbacks. Further, environmental improvements for the more inbred population might also improve its fitness. However, more severe inbreeding can result in negative population growth and an inevitable trajectory towards extinction. Thus, the existence of bottlenecked populations that still grow in abundance (Leberg, 1990; Adern and Lambert, 1997; Ramstad et al., 2013) cannot be used as evidence that inbreeding and loss of genetic variation do not have deleterious effects on population persistence: comparisons must be made of population growth rates (or other measures of fitness) of populations with different levels of inbreeding in the same environment (and not only at carrying capacity).

A8. Links between evolutionary potential and inbreeding depression

Franklin (1980) considered only evolutionary potential and quantitative genetic variation in the context of N_e = 500 (Jamieson and Allendorf, 2012, 2013). Since his theory involves the equilibrium between drift and mutation, it requires many generations. Consequently, evolutionary potential cannot be divorced from concerns about inbreeding depression in the long-term for two reasons. First, evolutionary change depends on the selection differential (see equation A2 above) and this is expected to decline over generations due to inbreeding depression affecting fitness in finite populations (Frankham et al., 2013). Causal links exist between F, N_e, and t in closed random-mating populations (equation 1 in main text), and these are linked to fitness because F is causally related to inbreeding depression for fitness.

Second, Soulé (1980) estimated that the time to extinction in closed random-mating populations was ~ $1.5N_e$ generations (based on the experience of animal breeders), due to the slow accumulation of inbreeding depression, while Reed and Bryant (2000) concluded it was approximately N_e generations in captivity. Thus, inbreeding depression can reduce long-term (evolutionary-scale) population persistence of populations with N_e = 500 (see also Frankham et al., 2013).

A9. Additional considerations of N_e required to maintain evolutionary potential in perpetuity

Subsequent to Franklin (1980) and Lande and Barrowclough (1987), Lande (1995) argued that only approximately 10% of new mutations are near neutral, meaning effectively that $V_m \sim 10^{-4}V_E$ and thus, the required N_e should be 5 000. Franklin and Frankham (1998) queried this new value, as most estimates of V_m come from long-term experiments where natural selection cannot be totally avoided (such that highly deleterious mutations are often removed). Further, alleles that are deleterious in one environment are often beneficial in another and contribute to

adaptation to changed environments (a major context for retaining evolutionary potential), as indicated by home site advantage (Hereford, 2009; Montgomery et al., 2010; Fraser et al., 2011)

The issue of deleterious mutations introduces consideration of reproductive fitness. For fitness characters (as opposed to peripheral traits), heritabilities are typically ~ 10-20% (Falconer and Mackay, 1996). Using h^2 of 20% (the mean value for fitness traits in wild birds: Frankham et al., 2010) and $V_m \sim 10^{-4}V_E$, yields $N_e \sim$ 1250 to retain evolutionary potential in perpetuity.

Another issue is that increases in mutational variance for life-history/fitness traits per generation (V_m/V_E) are lower than for peripheral traits (Houle et al., 1996). Further, those estimates of V_m/V_E were obtained in laboratory environments, while Rutter et al. (2010) reported estimates of $1\text{-}2\times10^{-4}$ for fitness of plants in the wild – much lower than are typically found in laboratory studies. If V_A/V_E is ¼ as assumed for fitness above, these yield N_e of 633-1250.

Additionally, fitness traits typically show considerable non-additive genetic variation within adapted populations (Crnokrak and Roff 1955). Consequently, $h^2/(1 - h^2) = V_A / (V_E + V_D + V_I)$, rather than V_A/V_E, where V_D and V_I are dominance and interaction variance (the sum of them representing the non-additive component; Falconer and Mackay, 1996). As non-additive variation ~ V_A for life-history traits (Crnokrak and Roff, 1995) and assuming $h^2 = 0.2$ for fitness in the wild (as found for birds), $V_A/V_E \sim 1/3$, while $h^2/ (1 - h^2) = ¼$, indicating that the approximation would underestimated N_e by 33%. Applying this adjustment to the Rutter et al., (2010) data, results in N_e of 844-1667.

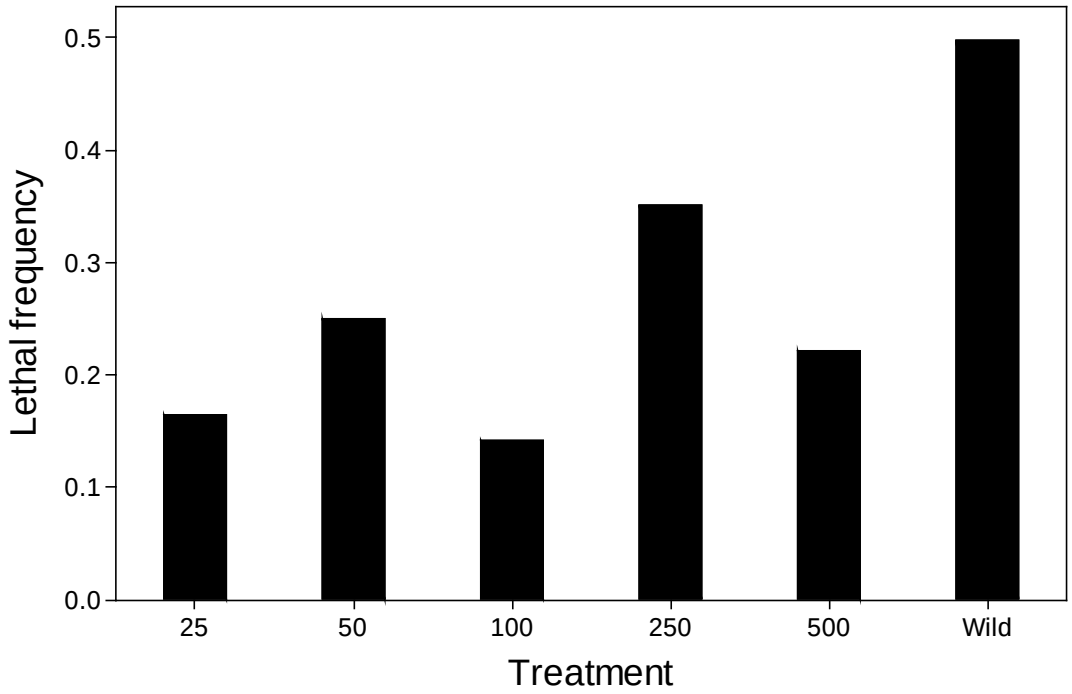

Fig. A1 Average lethal frequencies (Poisson-corrected) on second chromosomes in laboratory *Drosophila melanogaster* populations maintained at effective sizes of 25, 50, 100, 250 and 500 for 51-67 generations in comparison to that in a recently caught sample of their wild base population (Woodworth, 1996).

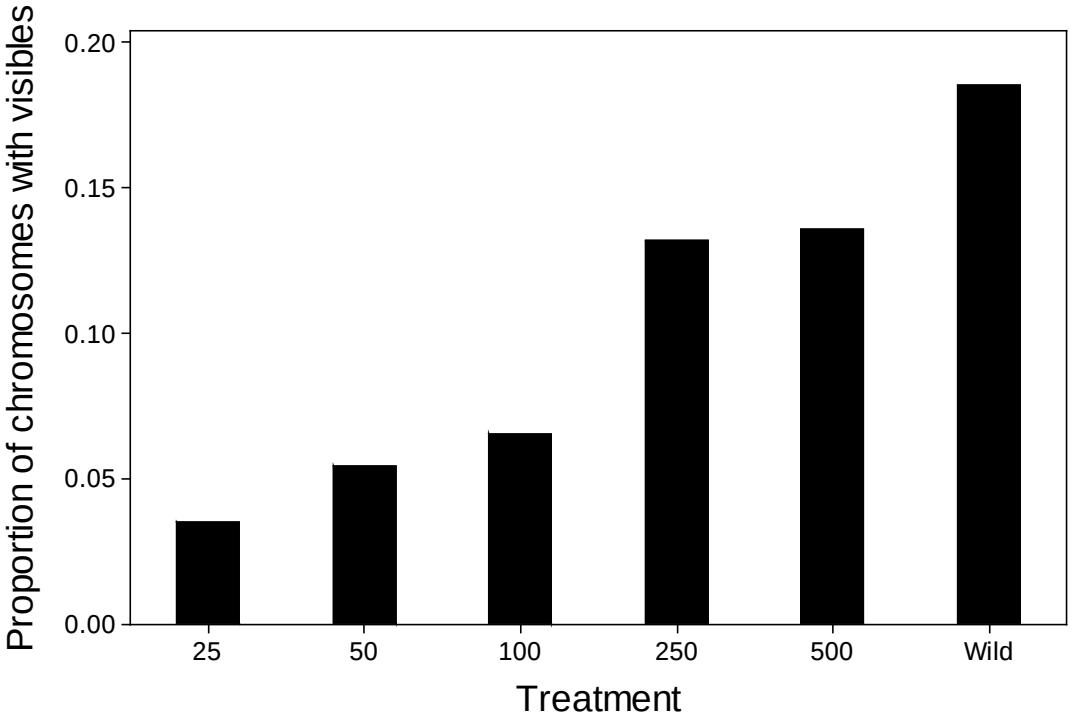

Fig. A2 Average proportions of second chromosomes with unique visible mutations in laboratory *Drosophila melanogaster* populations maintained at effective sizes of 25, 50, 100, 250 and 500 for 51-67 generations in comparison to that in a recently caught sample of their wild base population (Woodworth, 1996).

A10. Effects of methodology on N_e estimates

A range of methods are available to estimate effective population sizes, including (Frankham, 1995; Frankham et al., 2010; Luikart et al., 2010):

a. Demographic methods, based on variation in populations size over generations, variation in family sizes and unequal sex-ratios
b. Genetics methods based on loss of genetic diversity over generations, variation in allele frequencies, the extent of linkage disequilibrium, coalescence and
c. Equilibrium between drift and mutation over evolutionary times spans (Lynch, 2006).

The former two operate over timespans relevant to our conservation concerns (generally up to 50 generations), while the latter one is typically concerned with millions of generations, and requires estimates of mutation rates that are rarely available for the particular taxa. It is not considered further here, given its different basis and time frame. The demographic and genetic methods have similar expectations when both are used to obtain comprehensive estimates. In empirical comparisons of estimates obtained using different comprehensive genetic and demographic estimates, Frankham (1995) found little evidence of differences among methods. Further, Leroy et al., (2013) found little difference among comprehensive estimates obtained using four different methodologies: the means of estimates for 140 breeds of domestic animals all lay within the confidence intervals for the other comprehensive estimates. The main differences they found related to non-comprehensive versus comprehensive estimates.

Table A1

Consequences of low and high genetic connectivity among populations within species and results of empirical tests to compare the two scenarios

Characteristic	Connectivity		Results	References
	Low	High		
Populations	Isolated	Behave as a single population		
F_{ST}	> 0	~ 0	> 0.2 in > 25% of species	A10
Correlation heterozygosity-*N*	+ve	~ 0	usually +ve	1-3
Correlation fitness-*N*	+ve	~ 0	generally +ve	2,4
Genetic diversity and threat status	-ve	~ 0	-ve	5-7
Isolation by distance	common	weak/uncommon	fairy common	8-9
Correlation dispersal ability and F_{ST}	-ve	weak/zero	- 0.72	10

References: Section A10 contains detail of F_{ST} vales from a variety of taxa indicating the extent of restricted gene flow; 1. Frankham (1996); 2. Leimu et al. (2006); 3. Frankham (2012); 4. Reed and Frankham (2003); 5. Spielman et al. (2004); 6. Evans and Sheldon (2008); 7. Flight (2010); 8. Forbes and Hogg (1999); 9. Jenkins et al. (2010); 10. Bohonak (1999).

*

A10. F_{ST} data and restricted gene flow

Gene flow is restricted compared to random mating when $F_{ST} > 0$ and this will have growing impacts as F_{ST} increases (Wright, 1951), especially via inbreeding depression when some population fragments have small population sizes. $F_{ST} > 0.2$ is a stringent criterion for restricted gene flow that derives from Wright's work as the value where gene flow is too low to avoid fixations from drift with an island model at equilibrium where it corresponds to one effective migrant per generation, irrespective of population size (Wang, 2004). As F_{ST} was designed for loci with 2 alleles, it does not scale 0-1 with data for > 2 alleles (Hedrick 2005), and thus is typically biased downwards for microsatellite loci as they usually have > 2 alleles. Further, $F_{ST} = 0.2$ can be achieved by a population in equilibrium, or by one that previously had $F_{ST} = 0$ and then recently became fragmented with no gene flow. Consequently, $F_{ST} = 0.2$ does not necessarily mean a population has sufficient gene flow. Most estimates of F_{ST} are for > 2 populations (Bohonak, 1999) and so are average values across several populations; thus a value of $F_{ST} < 0.2$ does not mean that none of the populations is suffering restricted gene flow (under the $F_{ST} > 0.2$ criteria). F_{ST} values clearly also depend on the extent of sampling (Morjan and Rieseberg, 2004; Kisel and Barrowclough, 2010) and this will often be far from the complete species distribution, representing another reason why estimates are often biased downwards.

Nevertheless, compilations of F_{ST} values across species reveal a substantial proportion with FST > 0.2. The mean values of F_{ST} for a variety of major taxa in Table 14.4 of Frankham et al. (2010), reveal mean $F_{ST} > 0.2$ for mammals, reptiles, amphibians, selfing plants, and mixed mating animal pollinated plants, indicating restricted gene flow. We found five sources of information with individual F_{ST} values across a variety of species and sub-species. First, Bohonak's (1999) animal data reveal 25% of 134 vertebrate and invertebrate taxa with $F_{ST} > 0.2$ (for allozymes). Second, data in Latta (2004) showed 34% of 29 species of vertebrates, invertebrates and plants had $F_{ST} > 0.2$. Third, Leinonen et al. (2008) reported $F_{ST} > 0.2$ in 35% of 51 species of vertebrates, invertebrates, and plants. Fourth, Kisel and Barrowclough (2010) reported 41% of 194 species of vertebrates, invertebrates and plants with $F_{ST} > 0.2$ (excluding studies with only one locus and choosing studies with greater range, more populations and more loci when there was more than one study for a species and including data on allozymes, microsatellites and other DNA markers). Fifth, Alsos et al. (2012) reported $F_{ST} > 0.2$ in 93% of 27 northern plant species in the arctic and alpine zones of the Northern Hemisphere. As the above data might not represent random samples of taxa, we have partitioned the studies with the largest numbers of species into vertebrates, invertebrates and plants (Table A2). The largest samples revealed 26% of vertebrates, 29% of invertebrates and 55% of plants with $F_{ST} > 0.2$. Consequently, many species have limited gene flow among population fragments, even using this stringent and often downwardly biased measure. However, there is need for a comprehensive unbiased study of the extent of gene flow restriction across populations for a diverse range of species.

Fragmentation effects on quantitative traits (more likely to represent adaptive variation) are stronger than those for 'neutral' variation, as indicated by $Q_{ST} > F_{ST}$ (Latta, 2004; Leinonen et al., 2008, but see caveats in Whitlock, 2008 and Pujol et

al., 2008), indicating that the data above underestimate the evolutionary consequences of restricted gene flow even further.

Table A2
Percentages of species with F_{ST} > 0.2 in various major taxa

Major taxa	F_{ST} > 0.2	# species	References
Vertebrates	25.9%	54	1
Invertebrates	28.6%	105	2
Plants	55.0%	111	1

References: 1. Kisel and Barrowclough (2010); 2. Bohonak (1999).

A11. Genetic issues and the IUCN Red List categorisation system

Whilst there is a clear (implicit) intention to include genetic factors in the IUCN Red List categorisation system, the logic used is rather opaque and appears questionable. Under Criterion C, the population size thresholds (that trace to N_e values in Mace and Lande, 1991) must be combined with continuing decline. However, it is under these circumstances that genetic impacts on population persistence are least, as deterministic forces can drive species to extinction before genetic factors have time to impact them (Lande 1988; O'Grady et al., 2006). If the intent was to combine genetic factors with a variable that amplified their impacts, then high variation in population size would be more appropriate, as this reduces N_e and leads to greater inbreeding depression and accelerated loss of genetic diversity. Empirical data shows that species with high variation in population size require higher MVPs, as expected for both genetic and demographic reasons (Thomas, 1990), but this is not considered within Criteria C and D (it appears in B (iii) that involves habitat area).

Genetic factors have their greatest impacts in populations with relatively stable, non-declining sizes (conditions arising from non-genetic factors) (O'Grady et al., 2006), as such populations/species are typically not driven to extinction before genetic factors can impact them (Spielman et al., 2004). IUCN Red List Criterion D encompasses stable population sizes, but its intended relation to genetic factors is unclear. While species that recover from fluctuations in population size due to non-genetic factors bear no further adverse consequences, adverse genetic impacts are not 'healed' except by outcrossing, because inbreeding depression and loss of genetic diversity accumulate over generations in a ratchet-like manner in closed populations. For example, 97.5% of *Drosophila* populations with stable population sizes (N_e = 10) under benign captive conditions were extinct after 62 generations, and 61% of replicate populations with N_e = 20 were extinct after 60 generations (Reed et al., 2003), while no non-inbred population of these sizes would be extirpated under the same conditions. As extinction risks assessed by PVAs and the IUCN Red List categorisations exhibit only a modest rank correlation (r_S = 0.37, P = 0.003; O'Grady et al., 2004), revision of genetic consideration in the IUCN Red List categorisations would likely improve the relation between the two.

A12. A preliminary assessment of the impacts of doubling population size thresholds in the IUCN Red List categorization system on the frequencies of different threatened species categories.

We expect that a doubling of the population size thresholds in the IUCN Red List categorization system would increase the number of species listed in each of the threatened categories, but by how much needs to be determined by empirical evaluation. An assessment on 28 taxa in Table 15.2 of Frankham et al. (2010) using 100/1000 rather than 50/500 resulted in the changes shown in Table A3 below. Species already in the Critically Endangered category (12) cannot change, because the re-assessment can only move species into a higher threat category and they are already in the highest category and another had population size data that was insufficiently precise to allow categorisation. Further, another three species were categorised as CE under 50/500 based cut-offs in our exercise (Table A3).Thus, 4/12 (33%) of the species that could potentially change did move. Since the table did not contain any non-threatened taxa, our assessment does not indicate how many species would move from Lower Risk-Near-Threatened into the Vulnerable category, only movements of VU and EN species: however, we might expect these movements to be generally indicative.

Table A3

Changing IUCN threat classification for 7 of the 28 species listed in Frankham et al. (2010) Table 15.2 for which a new category is possible. Listed are the common and scientific name; IUCN RL is the threat category of the species from the 2013 Red List (based on multiple criteria), where VU = vulnerable, EN = endangered and CE = critically endangered. *N* is the estimated number of mature individuals. IUCN-C is the threat categorisation if based on the population-size thresholds for IUCN criterion C alone. N_e revision is the updated classification based on the updated N_e population-size thresholds for criterion C, as suggested in this paper.

				Criterion C N-threat	
Common Name	Species	IUCN RL	*N*	IUCN-C	N_e revision
Ethiopian wolf	*Canis simensis*	EN	440	EN	CE*
Northern Atlantic right whale	*Eubalaena glacialis*	EN	350	EN	CE*
Lord Howe Island woodhen[a]	*Gallirallus sylvestris*	EN	180	CE	CE
Palo Verdes blue butterfly[b]	*Glaucopsyche lygdamus*	E (USA)	200	CE	CE
Whooping crane[a]	*Grus Americana*	EN	<250	CE	CE
Pink pigeon	*Nesoenas mayeri*	EN	260	EN	CE*
Komodo dragon	*Varanus komodoensis*	VU	<3,000	VU	EN*

Footnotes: *Species that definitely change threat category under the revised N_e population-size revision suggested in this paper. For the other species: a. IUCN threat classification is lower than expected under the population-size threshold of criterion C because this species is stable or not heavily fragmented. b. Original classification of this subspecies (*Glaucopsyche lygdamus palosverdesensis*) is a regional (USA) not global (IUCN) rank.

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