



## Perspective

# Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation



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## ABSTRACT

Genetic rescue – ameliorating inbreeding depression and restoring genetic diversity of inbred populations through gene flow – is valuable in wildlife conservation. Empirically validated recommendations for genetic rescue supported by evolutionary genetics theory advise maximizing genetic diversity in target populations. Instead, recent papers based on genomic studies of island foxes, Isle Royal wolves, and simulation modeling claim it would be preferable to minimize introduction of harmful variation by avoiding genetic rescue altogether or by selecting partially-inbred sources presumed to have fewer harmful alleles. We examined the assertions and evidence underlying these new recommendations. The claim that long-term persistence of a few small inbred populations invalidates the small population paradigm commits the survivorship fallacy by ignoring population extinctions through inbreeding. The claim that island foxes show no inbreeding depression conflicts with elevated levels of putatively harmful alleles, low fecundity, and island-specific disease susceptibilities. The claim that the history of Isle Royale wolves represents likely outcomes of genetic rescues using immigrants from larger source populations is invalid: the unplanned addition of a single male to an inbred population capped at ~25 individuals does not represent sound genetic rescue. The simulations in Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print) apply several unrealistic assumptions and parameter distributions that disfavor large, outbred sources for genetic rescue. Accordingly, the simulations' conclusions conflict profoundly with those of > 120 meta-analysed real datasets, and do not overturn current empirically validated recommendations to maximize genetic diversity in the target population.

## 1. Introduction

The inevitable loss of genetic variation from small isolated populations increases their risk of extinction due to inbreeding depression and reduced ability to adapt to environmental change. Many of these population fragments can potentially be saved from extinction by genetic rescue: augmentation of genetic diversity that reduces inbreeding depression, increases population size and ability to evolve (Frankham, 2015, 2016; Whiteley et al., 2015). Genetic rescue enhances fitness and population growth, as long as non-genetic drivers of decline are also managed effectively (Tallmon et al., 2004; Bell et al., 2019; Ørsted et al., 2019; Hemphill et al., 2020).

There is ample evidence (Frankham et al., 2017) that loss of genetic

variation increases extinction risk as part of the 'small population paradigm' (Caughley, 1994). However, a few inbred wildlife populations, including island foxes (*Urocyon littoralis*), are reputed to have persisted for long periods in isolation (Coonan et al., 2010; Reed, 2010; Hofman et al., 2016). Some authors claim that this challenges the small population paradigm and question the importance of genetic variation for population persistence and the need for genetic rescue in some situations (Robinson et al., 2016, 2018).

This purported challenge to the richly supported small population paradigm could have a major, unwarranted influence on the application of genetic management of small and isolated populations of threatened species, either hindering it: 'the island fox illustrates a scenario in which genetic restoration through human-assisted gene flow could be a

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counterproductive or even harmful conservation strategy.’ (Robinson et al., 2018) or changing its implementation: ‘the idea that founders from historically isolated populations should be selected to mitigate the risk of future inbreeding depression stands in contrast with the conventional wisdom of selecting individuals with the most diversity from nearby populations. However, this alternative should be considered as a potentially more successful strategy for preserving species consisting of small and isolated populations’ (Robinson et al., 2019), and ‘Our findings challenge the traditional conservation paradigm that focuses on genetic diversity in assessing extinction risk in favor of a new view that emphasizes minimizing deleterious variation.’ (Kyriazis et al., 2019 non-peer-reviewed pre-print). At the time of writing, that non-peer-reviewed pre-print had been cited as evidence regarding the best approaches to genetic management at least 8 times in peer-reviewed articles (Supplementary material) and highlighted in the science media (Pennisi, 2019). The influences of these papers on wildlife management are already evident: for example, de Manuel et al. (2020) concluded in relation to very low genetic diversity in the Gir lion population ‘one future action to consider would be boosting their genetic diversity through outbreeding with such lions. However, in this regard, we are fully aware that this strategy would be both politically challenging, and, in light of recent observations on the effect of genetic introductions in the Isle Royale wolves (37), not guaranteed to be beneficial, so such decisions should not be taken lightly.’ (reference 37 is Robinson et al., 2019).

It is important that genetic management be applied effectively when required, because most species have fragmented distributions resulting from human activities, many with small isolated populations suffering low genetic diversity, inbreeding and consequent reduced fitness and an elevated risk of extinction (Frankham et al., 2017). Established guidelines for genetic rescue recommend using source populations that have a low risk of causing outbreeding depression, are genetically diverse and have the lowest mean kinship (co-ancestry) with the population needing rescue, because this minimizes inbreeding in the rescued population, maximizes enhancement of genetic diversity, which facilitates adaptation to changing environments, and is empirically extremely successful (Frankham, 2015; Whiteley et al., 2015; Frankham et al., 2017). The suggested opposing strategy of introducing individuals from smaller, more-inbred populations is cast as minimizing the introduction of harmful variation at the expense of lower genetic diversity and higher inbreeding in the recipient population. This proposal is based on the idea that in smaller, partially inbred populations highly harmful mutations would have been removed by ‘purging’ (selection against harmful variants, particularly recessive and partially-recessive alleles) (Robinson et al., 2018, 2019; Kyriazis et al., 2019 pre-print).

Our objectives in this perspective are to assess claims that:

- ‘The long-term persistence of island foxes despite their small population sizes and increased genetic load presents a challenge to the small-population conservation paradigm’ (Robinson et al., 2016).
- island foxes show no inbreeding depression and the long-term persistence of island fox populations ‘provides a model for preservation of small fragmented populations’ (Robinson et al., 2018).
- the history of the Isle Royal wolf population is a good model for the probable results of genetic rescue using outbred immigrants from a large population (Robinson et al., 2019; Kyriazis et al., 2019 pre-print).
- immigrants from small ‘historically isolated populations’ (Robinson et al., 2019) or ‘small or moderate size populations’ (Kyriazis et al., 2019 pre-print) will have more beneficial genetic rescue impacts when introduced into small, isolated inbred populations than will immigrants from larger, more outbred populations, due to the prior purging of highly harmful alleles from smaller populations.
- computer simulations support the use of small, presumably purged, populations as sources for genetic rescue (Robinson et al., 2019; Kyriazis et al., 2019 pre-print).

These five claims would have far-reaching implications for biological conservation if valid, and are assessed below in Sections 2–6, respectively.

## 2. Persistence of a few inbred populations does not invalidate the small population paradigm

Proposed cases of long-term survival of populations with low genetic variation may have overlooked past gene flow, as for the Isle Royale wolf population (Hedrick et al., 2014). Although Robinson et al. (2018) did not detect gene flow among Californian Channel Island fox populations with their sample size of a single fox per island (except two from one island), studies using ~20–40 individuals per population found clear evidence of gene flow, inconsistent with long-term isolation (Hofman et al., 2015; Funk et al., 2016). Accordingly, past genetic rescue has likely contributed to the persistence of island foxes. Further, apparent persistence of small, isolated populations may in fact involve cryptic decline or extinction masked by re-colonization (Grosser et al., 2016; Rawlence et al., 2017).

Foxes are thought to have colonized the northern Californian Channel Islands ~7100–9200 years ago, probably by rafting or introduction by Native Americans, followed by human translocation of foxes from the northern to southern Channel Islands (Hofman et al., 2015, 2016). The archeological record of persistent interest in these foxes by Native Americans, including people provisioning and burying them, the purported introduction of foxes from Santa Catalina Island to San Clemente Island by ranchers in 1875, and foxes kept as pets in the 20th century (Hofman et al., 2015), all suggest mechanisms for inter-island fox movements. Many other cases of supposed long-term isolated populations demand re-examination with powerful methods, ideally with historical and modern samples.

Even in true cases of persistence of isolated, inbred populations, we see only the few that survived, not the many that went extinct (Steadman, 1995; Frankham, 1997, 1998). Thus, the claim of Robinson et al. (2016) that the long-term persistence of a few inbred wild populations challenges the small population paradigm commits the survivorship fallacy (Shermer, 2014), akin to arguing that the survival of a few heavy smokers to advanced age shows that smoking does not increase the risk of lung cancer.

Like octogenarian chain-smokers, some small inbred populations may survive through luck of the genetic draw in favorable environments (Reed, 2010). The magnitude of fitness depression following inbreeding varies among species and even among populations, families, and individuals (Frankham et al., 2017). Thus, although most highly inbred populations go extinct, theory predicts a few lucky survivors, as well-supported by experimental evidence. In classic experiments on inbreeding in guinea pigs (*Cavia porcellus*), 30 of 35 inbred lines went extinct or declined so severely that breeding was discontinued (Wright, 1977); similar results occur when domesticated species and experimental model organisms are inbred (Frankel and Soulé, 1981; Ralls and Ballou, 1983; Frankham, 1995).

Notwithstanding their occasional persistence, inbred populations are consistently less fit than outbred ones. For example, Wright’s surviving inbred guinea pig lines were consistently inferior in fitness compared to controls (Wright, 1977). Similarly, outbred animals and  $F_1$  hybrids routinely surpass inbred strains of mice in key reproductive traits (Silver, 1995). In *Drosophila*, all 31 of 80 populations maintained with effective population size  $N_e = 20$  that survived extinction and reached an inbreeding coefficient ( $F$ ) of 0.8 had lower fitness than their outbred source (Reed et al., 2003). Similar conclusions apply to wild populations in captivity and nature (Ralls and Ballou, 1983; Crnokrak and Roff, 1999). Even inbred populations apparently doing well are likely suffering inbreeding depression and reduced ability to adapt to rapidly changing environmental conditions (Frankham et al., 1999; Ørsted et al., 2019).

### 3. Island foxes probably suffer from inbreeding depression and are not an appropriate model for the management of historically fragmented populations

Absence of evidence is not evidence of absence. Claims such as those of Robinson et al. (2018) that populations show no inbreeding depression would be more credible if they demonstrated appropriate design and power to register any inbreeding depression present. Detecting inbreeding depression requires comparisons of inbred and non-inbred individuals in similar environments, with sufficient estimation of inbreeding levels, variance in inbreeding, and sample sizes (Charlesworth and Willis, 2009; Miller and Colman, 2014). Inbreeding depression does not occur in every trait in all populations, but it accumulates over lifespans (Szulkin et al., 2007; Huisman et al., 2016; Niskanen et al., 2020). Accordingly, the best evidence about inbreeding depression requires data on total lifetime fitness, with a sufficiently powerful assay. Lacy (1997) concluded that no species of mammal has been found to be unaffected by inbreeding depression, when suitably conducted tests were applied.

The claim that island foxes do not exhibit inbreeding depression is based on rarity of congenital skeletal defects (Robinson et al., 2018). While these may be a symptom of severe inbreeding depression (as they may be for Isle Royale wolves, Robinson et al., 2019), such peripheral characters are less-sensitive indicators of inbreeding depression than are traits closer to total fitness, such as survival and fecundity (Falconer and Mackay, 1996; DeRose and Roff, 1999). Traits closer to fitness present evidence that island foxes probably do suffer inbreeding depression relative to the outbred ancestral mainland gray fox (*Urocyon cinereoargenteus*), accompanied by elevated levels of inferred harmful sequenced alleles (Robinson et al., 2016, 2018). Island foxes have an extremely small average litter size of 1.8 pups compared to ~4 in the gray fox (some of the difference might be due to smaller body size of the island foxes), and island-specific disease-susceptibilities such as ear tumors in Catalina Island foxes and amyloidosis in San Clemente Island ones (Fritzell and Haroldson, 1982; Clifford et al., 2007; Vickers et al., 2015; Gaffney et al., 2016).

Contrary to the view that ‘Genetic rescue should not be used as a conservation technique for historically small populations, as on many islands, without compelling evidence of inbreeding depression.’ (Robinson et al., 2018), we argue that the need for genetic rescue should be assessed by weighing the risks and benefits of alternative actions (Frankham et al., 2017; Ralls et al., 2018; Liddell et al., 2020). Given that studies with adequate sample size and assessments of multiple fitness components show inbreeding depression to be nearly universal (Angeloni et al., 2011; Niskanen et al., 2020), compelling evidence for a lack of inbreeding depression should be required before the decision is made to allow a threatened population to remain severely inbred (Ralls et al., 2018). For many species it will be impossible to demonstrate inbreeding depression within a suitable timeframe and/or with available resources. Waiting to act until inbreeding depression has been demonstrated will increase extinction risk because of low fitness and failure to provide adaptive genetic variation needed to cope with changing environments.

The highly questionable conclusion of no inbreeding depression in island foxes (this section), combined with the uncertainty that a given small population really was isolated at small size for very long periods and the survivorship fallacy (Section 2), invalidate the assertion that ‘the independent long-term persistence of all six island fox populations for thousands of years suggests that small populations on the order of a few hundred individuals can persist over evolutionary timescales and provides a model for the preservation of small fragmented populations’ (Robinson et al., 2018).

### 4. The history of the Isle Royale wolf population is not an appropriate model for a genetic rescue

Wolves, probably one female and two males, colonized Isle Royale in Lake Superior, Michigan, USA from the mainland in 1949 or 1950 (Adams et al., 2011), when the channel between mainland and island froze. The 544 km<sup>2</sup> island can support only ~24 wolves with an effective population size of ~3.8 (Adams et al., 2011). By the late 1990s, the island population was extremely inbred. Mainland male M93 migrated to the island in 1997. His reproductive fitness was so much higher than that of his inbred competitors that only 2.5 generations later he was related to every wolf on the island and responsible for 59.4% of the population's ancestry. However, the wolf population did not increase in size despite initial increased individual fitness, because space was limiting, and this male arrived during a period of low prey abundance. The population subsequently declined precipitously with increasing levels of inbreeding (Hedrick et al., 2014, 2019).

This rise and fall of this wolf population is entirely to be expected from theory and empirical cases. If populations remain small and isolated, ongoing genetic rescue is necessary to keep inbreeding below harmful levels (Hedrick and Fredrickson, 2010; Frankham et al., 2017). Nonetheless, some authors have suggested that genetic rescue from genetically diverse populations might contribute to population declines: ‘individuals derived from historically large populations, such as the mainland source population for Isle Royale wolves, carry a greater burden of strongly deleterious recessive mutations in the heterozygous state relative to individuals from historically smaller populations. Strongly deleterious recessive alleles carried as heterozygotes within the genomes of founders or immigrants quickly become homozygous through inbreeding in small isolated populations, resulting in inbreeding depression’ (Robinson et al., 2019). Rather than ask why the wolf population declined under severe recent inbreeding, we should instead ask why it persisted in the first place, given its very low effective population size. Past immigration is the likely explanation: the estimated ~1 migrant per generation of gene flow from the mainland to the island in past years could explain the persistence of the island population up to the arrival of M93 in 1997 (Hedrick et al., 2014). The probability of an ice bridge forming in a given winter has declined from 0.67 during the 1960s to only 0.19 in the decade to 2014. No additional wolves crossed to the island since the arrival of M93, so the population became genetically isolated (Vucetich and Peterson, 2014).

The Isle Royale wolf population does not represent a good model for a well-conducted genetic rescue. It is highly unlikely that an isolated population with a carrying capacity of ~24 wolves can be demographically sustainable for long periods (Goodman, 1987), and the effective population size of only ~3.8 is far lower than that at which harmful inbreeding ensues in virtually all populations that usually outbreed (Lacy, 2000; Frankham et al., 2014). Whatever genetic variation was carried by M93, his arrival would have been insufficient for persistent genetic rescue of the tiny, isolated Isle Royale population (Hedrick et al., 2014, 2019), as would the arrival of any other single immigrant. If the population is to be maintained, it will be necessary to introduce new wolves periodically to the island (Hedrick et al., 2014; Robinson et al., 2019).

In contrast to the low ceiling on wolf population growth on Isle Royale, most common definitions of genetic rescue emphasize that gene flow results in increased fitness reflected in positive demographic responses, and all implicitly or explicitly rely on gene flow resulting in genetic variants being added to a population (Hufbauer et al., 2015; Whiteley et al., 2015; Bell et al., 2019). The Kyriazis et al. (2019 pre-print) simulations did not meet these core conditions of a genetic rescue: the population could not grow, and the extent of admixture in the generations following introduction of animals from another population was untested. In addition to time to extinction, reporting of data on population trajectories, admixture proportions, heterozygosity, and allelic diversity in the simulated ‘rescued’ populations would have

helped determine whether gene flow was actually effective in the modeled populations.

The history of the Florida panther (*Puma concolor*) after introduction of eight females from Texas into the remaining population of fewer than 30 panthers in 1995 provides a better model of the probable results of a genetic rescue using immigrants from outbred sources (Johnson et al., 2010). Unlike the Isle Royale wolves, the population was able to colonize unoccupied habitat. Genetic heterozygosity doubled, survival and fitness improved, the frequency of kinked tails and undescended testes declined significantly, and panther numbers increased more than threefold by 2007 and reached 230 by 2017 (Flescher, 2017). Although the population is still doing well and continuing to expand its range, managers recognize that it may require additional genetic rescues in the future, as well as access to additional habitat to support a larger population (van de Kerk et al., 2019). The success of the Florida panther genetic rescue using immigrants from a large outbred population is not an isolated case but an exemplar of the norm (Frankham, 2015).

## 5. Theory and a large body of empirical data support the preferential use of large source populations for genetic rescue rather than smaller ones

### 5.1. Benefits of using immigrants from large source populations

Introducing individuals from large, non-inbred source populations is, on average, about twice as effective at improving fitness and genetic diversity as introducing them from small inbred populations (Frankham, 2015) and it will be longer before additional gene flow is required when more diverse sources are used (Frankham et al., 2017).

Overtaking the large body of evidence supporting current genetic rescue guidelines based on increasing genetic diversity and reducing inbreeding would require very solid counter-evidence. But there is virtually no empirical support for claims that using immigrants from 'historically small' populations (Robinson et al., 2018) will result in more beneficial impacts than will using immigrants from large, outbred populations (below). A primary basis for those ideas is the simulation modeling of Robinson et al. (2018, 2019), which modeled changes in harmful alleles but not demographic or fitness responses, and Kyriazis et al. (2019 pre-print), which depend on problematic assumptions, omissions, and parameter settings, some of which are assessed below in Section 6. In contrast to the assertions made from these computer simulations, there is abundant evidence from wild and experimental populations of organisms that using outbred individuals from large, genetically variable populations for genetic rescues usually has better outcomes than using inbred ones from smaller populations (meta-analysis of Frankham, 2015).

The amount of inbreeding depression depends on the amount of inbreeding. This inbreeding may arise from mating between close relatives (e.g. brother-sister or parent-offspring) or more slowly in isolated small populations where inbreeding is unavoidable by genetic drift (Falconer and Mackay, 1996). After crossing an inbred population with another one, reversal of inbreeding is greater in the  $F_2$  (second filial) and subsequent generations using outbred than inbred immigrants (Frankham, 2015). For example, if a fully inbred population ( $F = 1$ ) is crossed to a distinct fully inbred population, the inbreeding coefficient in the  $F_2$  is 0.5, whereas if the cross is to an outbred population ( $F = 0$ ), the resulting level of inbreeding is  $F = 0.25$ , 50% lower (Morgan et al., 1998). Consistent with this numerical treatment, a meta-analysis of 120 genetic rescues revealed a median benefit of 113.6% for a variety of fitness measures using outbred immigrants compared to less than half as much using inbred ones (Frankham, 2015). An experimental study evaluating 2455 crosses between 12 population pairs across a range of locations and environments of a daisy, *Rutidosis leptorrhynchoides*, found the main determinant of progeny fitness following population augmentation to be gene flow from large, genetically

diverse populations (Pickup et al., 2013).

In addition to improved individual fitness, there are also clear benefits of using outbred rather than inbred immigrants to supply genetic diversity necessary to allow populations to adapt to rapidly changing environments caused by global climate change and other human impacts (Frankham, 2015; Frankham et al., 2017; Ørsted et al., 2019). Such adaptation can be very rapid (Binks et al., 2007; Mitchell et al., 2019).

Even if the arguments were correct that smaller populations are relatively purged of harmful variants, the benefits would have to be large enough to overcome the smaller reduction in inbreeding and introduction of less beneficial genetic variation. But there are grave doubts about the validity of the arguments about using purged sources, as follows.

### 5.2. Purging effects are usually modest, unpredictable, and associated with fixation of some harmful variants and loss of some beneficial variants

Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print) argue that smaller populations will have lower levels of highly harmful mutations than larger ones. While some purging effects have been detected in some small populations, meta-analyses and empirical studies present little evidence that purging improves the overall genetic health, fitness, and persistence of most small isolated populations (Ballou, 1997; Byers and Waller, 1999; Frankham et al., 2001; Boakes et al., 2007; Leberg and Firmin, 2008). This is because purging:

- will be weaker for less-harmful variation, which is abundant in the genome and together has large negative effects on fitness. In smaller populations, selection efficiency is hampered by strong random drift (Frankham et al., 2014 Appendix A5)
- is 5–10 times less effective when the purging is caused by drift (as in Robinson et al., 2016, 2018, 2019; Kyriazis et al., 2019 pre-print) than by non-random mating (Glémin, 2003). Only highly recessive mutations are purged by drift in small populations, whereas purging by non-random mating (inbreeding) is effective for loci of any dominance level in large but not small populations
- typically removes some harmful alleles in small isolated populations but leads to higher frequencies or fixation of others (Hedrick, 1994; Grossen et al., 2020). For example, a dwarfism allele that is lethal when homozygous is at a frequency of ~9% in critically endangered California condors, which have a history of small population size (Ralls et al., 2000; Snyder and Snyder, 2000)
- removes genetic variation elsewhere in the genome, causing inbreeding depression at additional loci, reducing total population fitness, and ability to adapt to new environments (Charlesworth, 2013)
- does not work at all for loci subject to balancing selection, which contribute to fitness (Charlesworth and Charlesworth, 1987; Charlesworth and Willis, 2009; Bergland et al., 2014)
- may increase fitness in the environment under which the purging occurred but not in other environments (Bijlsma et al., 1999).

Experimental evidence indicates that purging effects are modest in their ability to reduce harmful genetic variants in ways that will promote persistence of small populations. An emerging approach is to compare frequencies and homozygosity of genomic variants of predicted negative fitness effects in presumably purged populations with those in non-purged populations. Some studies report more effective purging in more diverse populations (e.g. Ferchaud et al., 2018), and other studies conclude that small populations accumulate a greater load of harmful variants even if the most-harmful variants were subject to purging (e.g. Hamabata et al., 2019; Grossen et al., 2020). Likewise, the levels of putatively harmful variants in the observed genomic data were higher in island foxes than their mainland ancestors (Robinson et al., 2016, 2018). Robinson et al. (2016) concluded that 'small population



sizes have not resulted in more efficient purging of deleterious alleles, and have instead significantly increased the genetic load of the island populations', including for presumed strongly harmful loss-of-function (LOF) and missense alleles: 'Across all populations, island foxes had elevated homozygosity of derived alleles, with more than double the number of homozygous LOF and deleterious missense alleles compared to gray foxes' (Robinson et al., 2018).

The presumed fitness consequences of protein variants based on genomic data are likely to have some validity, although they are subject to many uncertainties demanding caveats (Koufopanou et al., 2015; He et al., 2016). Genomic elements other than proteins are more rarely assessed, yet have major fitness implications (Section 6 below). Although genomics has made it possible to suggest types of mutation that appear unconditionally harmful, these should be interpreted with caution: even loss-of-function mutations may be beneficial and adaptive (Monroe et al., 2018). It is still not possible to reliably predict the effects of most putative harmful mutations on the fitness of a wild population of non-model organisms, much less the combined effects of numerous harmful mutations in diverse environments (Henn et al., 2015; He et al., 2016). The level of harmful alleles in a population encompasses their number in the population, their mean number and distribution in individual genomes, population frequencies, degree of dominance/recessivity, and harmfulness when expressed phenotypically in a given environment. These characteristics change under different demographic histories in complicated fashions, and the distributions of parameters needed to predict the size of a population's genetic load, including selection coefficients, levels of dominance of harmful mutations, and rates of inbreeding are not well-understood even in the most comprehensively studied species, such as humans (Henn et al., 2015). In short, we do not know enough about the population genomic basis of fitness in most wildlife to accurately predict source populations with optimally low levels of harmful variation as required by the Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print) proposals (Harrisson et al., 2014; He et al., 2016). When methods of identifying harmful alleles improve, we assert that it will still be preferable to choose individuals with a minimal number of harmful alleles of large effect from large outbred populations rather than from small, presumably purged ones, because in the former the rest of their genomes will be more helpful to the target population. Assessing harmful variation has potential to be helpful in specific circumstances, if and when predictive methods are validated with fitness data (He et al., 2016). For example, they might be valuable for filtering out particularly unfavorable sources for genetic augmentation (Ferchaud et al., 2018).

In the context of genetic rescue, what matters for population persistence is the fitness and adaptive potential of the target population after introducing individuals from more- vs less-purged donor populations. Empirically, small, partially inbred populations have often not been effectively purged of the variation causing inbreeding depression: even populations with very low diversity usually continue to exhibit inbreeding depression when inbred further (Ballou, 1997; Dudash and Carr, 1998; Boakes et al., 2007; Mattila et al., 2012; Kennedy et al., 2014; Harrisson et al., 2019; Zilko et al., 2020). For example, the threatened New Zealand takahe bird (*Porphyrio hochstetteri*) showed an 88% reduction in recruitment of second-generation offspring for the progeny of full-sib matings relative to the progeny of unrelated pairs (Grueber et al., 2010), and isolated island populations of *Peromyscus polionotus* mice showed similar inbreeding depression as mainland comparators (Lacy et al., 1996). Even the long-term highly inbred cheetah (*Acinonyx jubatus*) suffers from inbreeding depression when further inbred in captivity (Hedrick, 1987).

The information above illustrates the great uncertainty around the effectiveness of purging to remove harmful variation. But unfavorable variants are only one side of the coin. Real populations also have beneficial alleles, which will be lost in small populations that purge harmful variation, because random genetic drift will limit the

effectiveness of selection (Charlesworth, 2013). The addition of beneficial variation to populations will be impeded under proposals that prioritize trying to minimize addition of potentially harmful variation at the expense of diversity, reducing potential gains in fitness and rapid adaptation to changing environments (Binks et al., 2007). Promoting beneficial genetic diversity is vital because environments are changing so rapidly that some species are no longer adapted to their current environments (Browne et al., 2019).

A proposal to purposely use small and medium populations presumed to be purged as sources of genetic variation in wildlife management would require at least reliable evidence that most purged populations are superior for genetic rescue compared to non-purged ones, including showing that chosen immigrants would have favorable genetic characteristics.

## 6. Problems and limitations of the simulation modeling

### 6.1. Description of the Robinson/Kyriazis modeling approach

Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print) simulated isolated island population scenarios using versions of the SLiM software package (Haller and Messer, 2019), using similar genetic models. SLiM allows for inclusion of an extensive range of demographic and genetic variables for modeling finite populations, offering valuable potential for projecting population futures and making conservation recommendations using available evidence. The modeling by Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print) involved genome-wide coding loci on realistic numbers of chromosomes with mutation, linkage, and recombination based on the dog genome, was directed at important questions, and was unusually sophisticated in some respects.

Best practices for simulation modeling and reporting include a clear description of the model so that others can replicate it, justification of the assumptions made, a sensitivity analysis of key assumptions and uncertainties, and validation of model results against empirical data (Brook et al., 2000; Ralls et al., 2002; Kettenring et al., 2006; Grimm et al., 2014). The modeling by Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print) contained unjustified, unrealistic values for some key parameters that pre-disposed them to produce biologically implausible results conflicting with theory and data from wildlife populations. Below we consider some genetic issues in the simulations that are particularly problematic.

### 6.2. All harmful mutations were modeled as completely recessive

All loci contributing to inbreeding depression were modeled as complete recessives, whereas partially recessive alleles are much more common in real organisms (Yang et al., 2017, plus below). The resulting exaggeration of the frequency of harmful recessive alleles in large populations will incorrectly disfavor those populations as sources for genetic rescue.

For selected loci, only additive and fully recessive options were modeled by Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print), while Robinson et al. (2016) modeled only additive loci. We will not consider additive loci further, because they do not contribute to inbreeding depression hence are irrelevant to assessing it (Falconer and Mackay, 1996), although they may provide beneficial alleles to the target population in a genetic rescue. Modeling all loci contributing to inbreeding depression as fully recessive is unrealistic because the overwhelming majority of harmful mutations are only partially recessive and have very different population genetic behavior than fully recessive ones (Charlesworth and Charlesworth, 2010; Manna et al., 2011; Caballero et al., 2016). The equilibrium frequencies of partially recessive alleles in very large populations are much lower than those for fully recessive ones (Crow and Kimura, 1970; Glémin, 2003; Hedrick and Garcia-Dorado, 2016). For example, the equilibrium frequency of a

harmful allele in mutation-selection balance for an autosomal locus with selection coefficient 0.01 and mutation rate  $10^{-5}$  (as in Robinson et al., 2018, 2019) is 0.0316 for a complete recessive but only 0.005 for a partial recessive with an average dominance of 0.2 (as observed empirically, Caballero et al., 2016). Further, partially recessive alleles are less affected by reductions in effective population sizes compared to fully recessive alleles, so they are less sensitive to purging (Crow and Kimura, 1970). Accordingly, modeling only fully recessive loci will lead to serious overestimation of frequencies of harmful recessive alleles in large populations and overestimate their sensitivity to purging.

### 6.3. Beneficial alleles and those under balancing selection were not modeled

No beneficial alleles (nor ones under balancing selection) were modeled, but they will be more common in larger populations and will have been subjected to more effective selection. This omission will incorrectly disfavor large populations as genetic sources.

Only harmful and neutral mutations were modeled, yet some mutations are beneficial, and some are maintained by balancing selection (Wheat et al., 2010; Hedrick, 2012; Bergland et al., 2014; Charlesworth, 2015). For example, Major Histocompatibility Complex immune system genetic variation in the island foxes that Robinson et al. (2018, 2019) modeled exhibits balancing selection (Aguilar et al., 2004). While a small proportion of loci appear to be subject to balancing selection, their individual contributions to inbreeding depression are typically much greater than those of loci subject to mutation-selection balance with similar selection coefficients. This is because inbreeding depression depends on heterozygosity, and loci subject to balancing selection have relatively high heterozygosities (Charlesworth, 2015). Thus, loci subject to balancing selection are considered to make an important contribution to fitness variation (Charlesworth, 2015; Sharp and Agrawal, 2018).

Accordingly, inclusion of some beneficial mutations is necessary for simulations of population futures to project fitness. Larger populations will have a greater number of high-frequency and fixed beneficial mutations than will smaller populations, and so larger source populations for genetic rescue will introduce more beneficial alleles than will smaller populations (Hill, 1982).

### 6.4. Variation in non-coding regions was not modeled

The simulation models considered only genetic variation in coding regions, yet much important genetic diversity lies outside coding regions. Large numbers of variants, particularly those with individually small selection coefficients and contributing to polygenic control of fitness, are much more readily retained in large than small populations: excluding such variants will incorrectly disfavor large populations as genetic sources.

In the two most-studied systems for quantitative genetic variation, humans and dairy cattle, a majority of genetic variation is due to variants outside coding regions. For example, 88% of SNPs associated with quantitative traits in humans were in introns and intergenic regions and likewise in dairy cattle (Hindorf et al., 2009; Ibeagha-Awemu et al., 2016). Such patterns are echoed in studies of fitness and adaptation in wildlife (overview in Harrisson et al., 2014). The SLiM software can simulate coding and non-coding regions (Haller and Messer, 2019). If non-coding variants are excluded from simulations, a major known source of fitness is unaccounted for. This should be acknowledged when making inferences and recommendations.

### 6.5. The importance of adaptive potential was not considered

Adaptive potential is important for fitness and persistence in real populations and is expected to be greatest in genetically diverse populations: excluding it will incorrectly disfavor large source populations.

Genetic rescue should result in improved ability to adapt to environmental change, but this is challenging to model and was not included in the simulations. Optimal genetic augmentation should provide genetic variability to enable evolutionary adaptation to changing environments. Omitting this from simulations could completely overturn conclusions about optimal genetic rescue strategies (Kearney et al., 2009; Ørsted et al., 2019; Razgour et al., 2019). When this cannot be included in simulations, it should be clearly acknowledged as unaccounted for.

## 7. Conclusions and recommendations

The current recommendation for genetic rescue, to choose a source population that will maximize genetic diversity in the target population, is well-supported by a large body of theory and empirical evidence, including planned experiments and successful genetic rescues. Guidelines for planning genetic rescues are provided in Frankham et al. (2017) and include decision-support tools for screening potential source populations for the risk of outbreeding depression if crossed with target populations. Contradictory recommendations by Robinson et al. (2018, 2019) and Kyriazis et al. (2019 pre-print) derive from simulation modeling that does not account for important realities, does not accord with empirical data from wildlife, experiments and evolutionary genetics, and are thus insufficiently supported to overturn current practices.

We offer the following recommendations to wildlife decision- and policy-makers and their advisors:

- Demogenetic simulations can be useful but inferences depend critically on assumptions, parameters and limitations. When simulations mismatch empirical data, the empirical data should be, by far, the preferred evidence for decision-making.
- Key assumptions, parameters and their justifications, and limitations of simulations should be easily found in scientific papers and reports, particularly those that make recommendations with real-world implications. Sufficient detail and data should be available such that a reader could re-create the analyses.
- A comprehensive test of genetic rescue should demonstrate that the management regime led to effective gene flow and increased genetic diversity, and evaluate benefits in population persistence or a robust proxy. Recommendations for evaluating the outcomes of genetic rescue attempts are provided by Robinson et al. (2020).
- Modeling harmful variation without also modeling beneficial variation is likely to lead to conclusions that smaller, more-inbred populations are better sources for genetic variation than they actually are. If beneficial variation is not included, inferences and recommendations should reflect this.
- The same applies to adaptive potential. It is important to consider this major factor in conservation planning because many populations are already maladapted to current conditions and have insufficient genetic diversity to respond (Browne et al., 2019).
- Genomic methods that seek to predict the fitness consequences of alleles are an important development, but they remain largely unvalidated and subject to considerable uncertainty, which should be made explicit.

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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