

1 High genetic diversity can contribute to extinction in small populations

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10 Abstract

11 Human-driven habitat fragmentation and loss has led to a proliferation of small and isolated
12 plant and animal populations that may be threatened with extinction by genetic factors. The
13 prevailing approach for managing these populations is to maintain high genetic diversity, which
14 is often equated with fitness. Increasingly, this is being done using genetic rescue, where
15 individuals from populations with high genetic diversity are translocated to small populations
16 with high levels of inbreeding. However, the potentially negative consequences of this
17 approach have recently been highlighted by the demise of the gray wolf population on Isle
18 Royale, which only briefly recovered after genetic rescue by a migrant from the large mainland
19 wolf population and then declined to the brink of extinction. Here, we use ecologically-
20 motivated population genetic simulations to show that extinction risk in small populations is
21 often increased by maximizing genetic diversity but is consistently decreased by minimizing
22 deleterious variation. Surprisingly, we find that small populations that are founded or rescued
23 by individuals from large populations with high genetic diversity have an elevated risk of
24 extinction due to the high levels of recessive deleterious variation harbored by large
25 populations. By contrast, we show that genetic rescue or founding from small or moderate-
26 sized populations leads to decreased extinction risk due to greater purging of strongly
27 deleterious variants. Our findings challenge the traditional conservation paradigm that focuses
28 on genetic diversity in assessing extinction risk in favor of a new view that emphasizes
29 minimizing deleterious variation. These insights have immediate implications for managing
30 small and isolated populations in the increasingly fragmented landscape of the Anthropocene.

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32

33 **Main text**

34 The prevailing paradigm in conservation biology prioritizes the maintenance of high genetic
35 diversity in small populations threatened with extinction (1–3). Under this paradigm, genetic
36 diversity is considered the primary determinant of fitness (4, 5), and the negative effects of
37 inbreeding are thought to be minimized by maintaining high genetic diversity. However, this
38 paradigm is challenged by the observation that some species, such as the Channel island fox,
39 can persist at small population size with extremely low genetic diversity and show no signs of
40 inbreeding depression (6, 7). This and other examples suggest that, rather than being mediated
41 by high genetic diversity, persistence for small populations may instead be enabled by the
42 purging of strongly deleterious variants, even when weakly deleterious variants increase in
43 frequency (6–10). In this study, we investigate the genetic factors mediating the persistence or
44 demise of small populations using population genetic simulations and demonstrate the
45 counterintuitive and potentially detrimental effects of high genetic diversity in small and
46 isolated populations. We argue that, in cases where populations are destined to remain small
47 and isolated, management strategies should aim to minimize deleterious variation rather than
48 maximize genetic diversity.

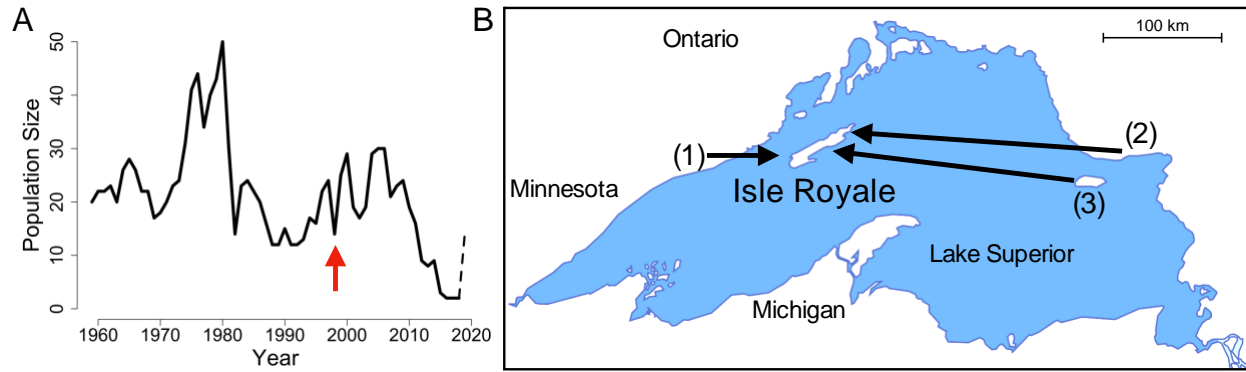
49
50 The motivating example for these simulations is the gray wolf population on Isle Royale, an
51 island in Lake Superior that has long served as a natural laboratory in ecology and conservation
52 biology (11–14). Following 70 years of isolation at a population size of ~25, the population was
53 driven nearly to extinction by severe inbreeding depression, with just two individuals remaining
54 in 2018 (14, 15)(Fig. 1A). Recent findings have suggested that the collapse of the population
55 was probably driven by the expression of recessive strongly deleterious alleles, which are
56 present in the mainland wolf population primarily in the heterozygous state, but were driven to
57 near-fixation in the island population after a mainland migrant ‘genetically rescued’ the
58 population by producing 34 offspring (Fig. 1A)(14–17). In response to the decline of the wolf
59 population, the US National Parks Service recently translocated 15 wolves to Isle Royale, which
60 were drawn from three nearby large source populations with the aim of maximizing the genetic
61 diversity of the new island population (Fig. 1B). However, the large ancestral population size of

62 these migrants implies that they likely carry high levels of recessive strongly deleterious
63 variation, potentially repeating the scenario that led to the initial population collapse. An
64 alternative approach for genetic rescue or reintroduction initiatives might instead target
65 historically smaller source populations with a history of purging of strongly deleterious variants,
66 or screen populations for individuals with low levels of strongly deleterious variation. This
67 approach could potentially alleviate problems with inbreeding depression by reducing the
68 number of strongly deleterious alleles in the small, isolated population. Given the growing
69 interest in genetic rescue as a management tool (8, 18, 19), such an approach could potentially
70 have wide-ranging implications for conservation.

71
72 The applicability of population genetic models to understanding extinction has historically been
73 limited by unrealistic assumptions that often ignore stochastic ecological factors and typically
74 do not consider both weakly and strongly deleterious variation (20–22). Here, we use a novel
75 population genetic simulation framework that combines ecologically-motivated models of
76 population dynamics with realistic genomic parameters (23)(SI) to assess how demographic
77 history, genetic diversity, and deleterious variation influence extinction risk in small
78 populations. Our simulations aim to capture the ecological factors that may contribute to
79 extinction in small populations, such as those observed in the Isle Royale population, by
80 incorporating the effects of demographic and environmental stochasticity, and natural
81 catastrophes (SI). Coupled with these stochastic population dynamics, we model a genome with
82 parameters reflecting that of a canine exome, including 20,000 genes and 38 chromosomes that
83 accumulate neutral and recessive deleterious mutations (SI). Using this framework, we first
84 explore the effect of the ancestral population size ($K_{\text{ancestral}} = \{1,000, 5,000, 10,000, 15,000\}$) on
85 time to extinction following a population contraction or founding event ($K_{\text{modern}} = \{25, 50, 100\}$)
86 (Fig. 2A). Although these parameter settings are motivated by the Isle Royale wolf population,
87 they also mirror those of many other classic examples of inbreeding depression and genetic
88 rescue, such as the Florida panther (24) and bighorn sheep (25).

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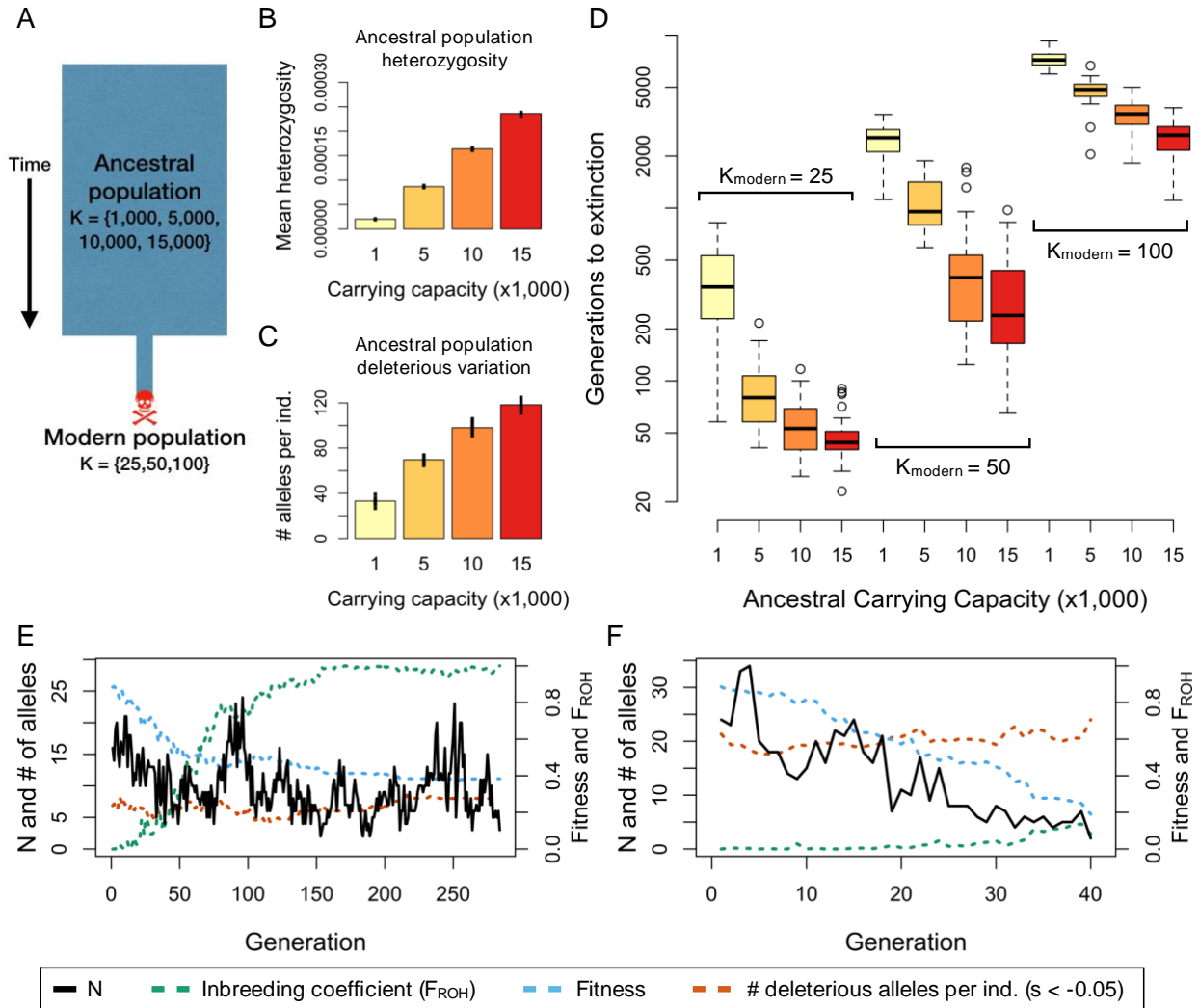
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92 **Figure 1: Isle Royale wolves.** (A) Population trajectory of the Isle Royale wolf population since 1960. Red arrow
93 denotes arrival of male migrant from mainland. Dashed line indicates population increase following translocation
94 of 15 wolves in 2018-2019. (B) Map of Lake Superior showing locations of three source populations for
95 translocation in 2018-2019. Source population localities include: (1) Grand Portage Indian Reservation (2
96 individuals), (2) Wawa, Ontario (3 individuals), (3) Michipicoten Island (8 individuals).
97

98 Our simulations demonstrate that large populations have higher levels of heterozygosity, as
99 expected (Fig. 2B), as well as a greater number of strongly deleterious alleles ($s < -0.01$) per
100 individual (Fig. 2C). Consequently, we observe a strong effect of ancestral population size on
101 time to extinction following a population contraction (Fig. 2D), with populations that were
102 historically large experiencing more rapid extinction. For example, given a modern carrying
103 capacity of 25, a population with an ancestral carrying capacity of 1,000 will go extinct on
104 average in 380 generations, whereas a population with an ancestral carrying capacity of 15,000
105 will go extinct in an average of 50 generations (Fig. 2B). This finding may be counterintuitive
106 given the prevailing view that small populations should be less fit due to an accumulation of
107 weakly deleterious alleles (21, 26, 27). The key driver of extinction that our simulations reveal is
108 that larger ancestral populations carry more recessive strongly deleterious alleles in the
109 heterozygous state (Fig. 2C). When the population contracts, elevated inbreeding exposes these
110 recessive deleterious variants as homozygotes, leading to a reduction in fitness and driving
111 extinction. The importance of recessive deleterious mutations in these simulations is further
112 supported by the absence of this effect of the ancestral population size when mutations are
113 assumed to have additive effects on fitness (Fig. S1-S2).

114



115

116 **Figure 2: Ancestral population size impacts the time to extinction of small populations.** (A) Schematic of the
 117 demography used in the simulations. (B) Mean heterozygosity of ancestral populations prior to contraction. (C)
 118 Average number of strongly deleterious alleles ($s < -0.01$) per individual in the ancestral populations prior to
 119 contraction. (D) Time to extinction for different combinations of ancestral and modern carrying capacities. Note
 120 that the y-axis is on a log-scale. (E) Representative population trajectory following contraction to a modern
 121 carrying capacity of 25 from an ancestral carrying capacity of 1,000 and (F) an ancestral carrying capacity of 15,000.

122

123 Examining individual simulation replicates provides insight into the dynamics of extinction in
 124 these populations (Fig. 2, E and F, Figs. S3-S5). Replicates with an ancestral carrying capacity of
 125 1,000 contain few strongly deleterious recessive variants after the contraction to a carrying
 126 capacity of 25, translating to a less severe loss of fitness as the population becomes inbred and
 127 longer persistence (Fig. 2E). By contrast, replicates with an ancestral carrying capacity of 15,000
 128 maintain high levels of strongly deleterious variation following contraction, leading to severe
 129 inbreeding depression and more rapid extinction (Fig. 2E). Even for replicates with identical

130 parameter conditions, we observe a high degree of variability in time to extinction, which
131 emerges from the interaction between the several forms of stochasticity in our model. For
132 example, populations that by chance have a low carrying capacity due to environmental
133 stochasticity immediately following the contraction quickly lose fitness due to inbreeding and
134 are often unable to recover, even after the carrying capacity subsequently increases. However,
135 populations that by chance had larger carrying capacities soon after contraction were better
136 able to purge their strongly deleterious variants, leading to longer persistence. The trajectory of
137 our simulated populations with an ancestral carrying capacity of 15,000 and modern carrying
138 capacity of 25 (Fig. 2F, Fig. S3) closely mirror that of the Isle Royale wolf population (Fig. 1A),
139 confirming that our simulations capture the genetic and ecological mechanisms relevant to the
140 decline of the population.

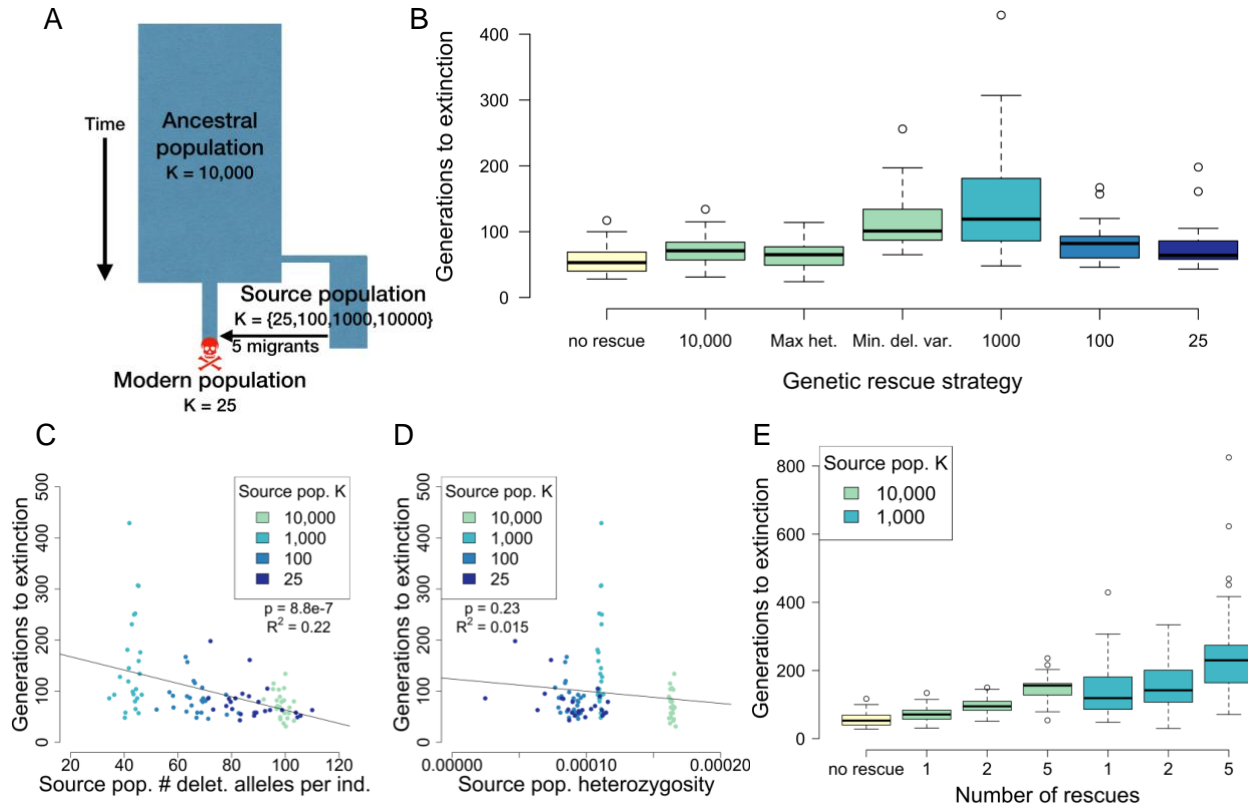
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142 Our simulations also demonstrate the importance of the carrying capacity of small, isolated
143 populations as determinant of extinction risk (Fig. 2D). Smaller populations tend to go extinct
144 most rapidly following a contraction, as expected given the higher levels of inbreeding in these
145 populations as well as magnified effects of ecological stochasticity (Fig. S6). Nevertheless, the
146 strong influence of the ancestral size was observed regardless of the post-contraction carrying
147 capacity, highlighting the importance of both recent and ancestral demography in determining
148 risk of extinction due to inbreeding depression.

149
150 We next examined how the levels of strongly deleterious variation and genetic diversity of the
151 source population influence the effectiveness of genetic rescue, defined here as the magnitude
152 of the increase in time to extinction. For these simulations, we fixed the ancestral carrying
153 capacity to 10,000 and modern carrying capacity to 25 and split off source populations from the
154 ancestral population prior to genetic rescue (Fig. 3A). We conducted genetic rescue after the
155 recipient population decreased in size to five or fewer individuals by translocating five
156 randomly-selected individuals from one of the following source populations: 1) a large source
157 population remaining at the ancestral size ($K=10,000$); 2) a moderate-sized source population
158 with long-term isolation ($K=1,000$ for 1,000 generations); 3) a small source population with

159 relatively recent isolation ($K=100$ for 100 generations); and 4) a very small source population
160 with very recent isolation ($K=25$ for 10 generations). These populations are highly variable in
161 their levels of genetic diversity and deleterious variation (Fig. S7), providing a range of
162 parameters to test how these factors influence the efficacy of genetic rescue.

163
164 Our results demonstrate that time to extinction following genetic rescue is highly dependent on
165 the source population demography and levels of strongly deleterious variation (Fig. 3, B and C).
166 For example, whereas genetic rescue from the moderate-sized source population ($K=1,000$) led
167 to a dramatic increase in mean time to extinction relative to the non-rescue scenario of 162%,
168 rescue from a large source population ($K=10,000$) resulted in a more modest increase of 28%
169 (Fig. 3B). Genetic rescue from small and moderately-inbred populations (Fig. S8) also resulted in
170 increases in mean time to extinction that exceeded that of the large source population (47%
171 increase for $K=100$, 34% increase for $K=25$) (Fig. 3B). Although conventional thinking would
172 suggest that the higher fitness of these larger populations (Fig. S8) would make them better
173 able to rescue a population (18, 28), individuals from these large populations carry many
174 heterozygous recessive deleterious mutations that quickly become homozygous in the recipient
175 population, resulting in more severe inbreeding depression. Indeed, these differences in time to
176 extinction following genetic rescue are predicted by the average number of strongly deleterious
177 alleles per individual in the source population (Fig. 3C), though not by source population
178 average genome-wide heterozygosity (Fig. 3D).

179
180 The finding that source population deleterious variation predicts the efficacy of genetic rescue
181 suggests that it may be possible to use genomic data to select individuals with low levels of
182 deleterious variation to use for genetic rescue. We explored this strategy by selecting the
183 individuals with the smallest number of strongly deleterious alleles ($s < -0.01$) from the large
184 source population ($K=10,000$) for rescue. This approach resulted in an increase in mean time to
185 extinction of 100% compared to the non-rescue scenario, a 57% increase relative to randomly
186 selecting individuals from the large source population (Fig. 3B). By contrast, when we selected
187 individuals with the highest genome-wide heterozygosity, we observed only a modest increase



188
 189 **Figure 3: Source population deleterious variation determines the effectiveness of genetic rescue.** (A) Schematic
 190 of the demography used in the simulation. (B) Time to extinction under different genetic rescue strategies.
 191 Numbers on x-axis denote source population carrying capacity; individuals selected with maximum heterozygosity
 192 or minimum number of strongly deleterious alleles ($s < -0.01$) were taken from $K=10,000$ source population. (C)
 193 Time to extinction following genetic rescue is negatively correlated with the number of strongly deleterious alleles
 194 ($s < -0.01$) per individual used for rescue. (D) Time to extinction following genetic rescue is not correlated with the
 195 heterozygosity of the source population. (E) Time to extinction as a function of the number of rescues from a large
 196 or moderate-sized source populations.

197

198 in time to extinction beyond the non-rescue scenario of 16%, a 10% decrease relative to
 199 selecting individuals at random (Fig. 3B). This result strikingly shows the potentially negative
 200 effects of trying to maximize genetic diversity in small populations, and highlights the role that
 201 genomic tools may be able to play in selecting individuals with low levels of deleterious
 202 variation for genetic rescue (29).

203

204 Lastly, we explored the effects of varying the number of migrants (1, 5, or 10) as well as the
 205 number of genetic rescue events (1, 2, or 5). These simulations show an approximately linear
 206 increase in time to extinction with increasing number of genetic rescues (Fig. 3E), suggesting
 207 that the efficacy of genetic rescue does not decrease with each additional rescue. However, we

208 observed minimal effects of the number of migrants, implying that only a few migrants may be
209 needed to achieve the beneficial effects of genetic rescue (Fig. S9).

210

211 Altogether, our simulations demonstrate the central importance of ancestral demography and
212 strongly deleterious variation in determining the risk of extinction due to inbreeding depression
213 in small and isolated populations. All else being equal, we find that populations that were
214 historically large have a much higher risk of extinction following a population contraction
215 compared to historically-smaller populations (Fig. 2D). These differences are mediated by the
216 higher degree of purging of strongly deleterious variants in populations of small or moderate
217 size (6, 8–10, 26). At first glance, this result may appear to be at odds with the thinking that
218 individuals from larger populations should be more fit due to stronger purifying selection
219 against weakly deleterious mutations (4, 21, 26, 27). The dynamics of extinction that our
220 simulations reveal is that, although large populations may have higher fitness, they also harbor
221 higher levels of heterozygous recessive strongly deleterious variation (Fig. 2, B and C). The
222 exposure of these strongly deleterious variants as homozygous in small populations can lead to
223 dramatic reductions in fitness and drive rapid extinction, well before ‘mutational meltdown’
224 due to weakly deleterious variants can occur (21). By demonstrating that population
225 contractions can result in levels of inbreeding severe enough to expose recessive strongly
226 deleterious mutations and that this effect is sufficient to decrease fitness and ultimately lead to
227 extinction, our simulations provide support for inbreeding depression being driven primarily by
228 recessive deleterious mutations rather than overdominance (8, 30). Although we did not model
229 overdominance in our simulations, empirical evidence overdominance as a driver of inbreeding
230 depression remains scarce, whereas recessive deleterious mutations are ubiquitous in diploid
231 outbreeding organisms (8, 30).

232

233 The influence of ancestral demography on extinction risk that our simulations reveal has wide-
234 ranging implications for assessing the threat of extinction due to inbreeding depression in
235 natural populations. Quantifying inbreeding depression in natural populations and predicting
236 the threat it poses to extinction represents one of the major challenges for conservation

237 biology, and it often remains unknown why some small populations appear to suffer from
238 inbreeding depression and others do not (8). Our simulations demonstrate that these
239 differences may be determined by the ancestral demography of a species. Consequently, we
240 suggest that information on ancestral demography, which is increasingly becoming accessible
241 using genomic data (31), could be more widely incorporated into extinction risk predictions.
242 However, our simulations also reveal that the fate of small populations is highly stochastic, and
243 that even under the same ecological and genetic parameters, time to extinction can vary
244 substantially (Fig. 2D). This result can help explain the observation that some populations can
245 persist at small size whereas others cannot, which may simply emerge from the stochasticity
246 inherent in the eco-evolutionary process.

247
248 Our simulations have especially important implications for choosing source populations and
249 individuals for genetic rescue or reintroduction, which are becoming increasingly important
250 tools for maintaining small and isolated populations under growing anthropogenic pressures (8,
251 18, 19). Our results demonstrate that the effectiveness of genetic rescue can be greatly
252 increased by targeting moderate-sized source populations with low levels of strongly
253 deleterious variation (Fig. 3, B and C), in contrast to existing recommendations to target
254 populations or individuals with high genetic diversity (18, 28). Strikingly, we found that genetic
255 rescue from large source populations with high genetic diversity was the least effective
256 strategy, even when compared to rescue from small and moderately-inbred populations, and
257 that targeting individuals with high genome-wide heterozygosity may in fact decrease the
258 effectiveness of genetic rescue (Fig. 3, B and D). However, our simulations also show that
259 genetic rescue from large source populations can be effective if individuals are screened for low
260 levels of strongly deleterious variation (Fig. 3B). Furthermore, we demonstrate that the
261 beneficial effects of genetic rescue may persist after multiple rounds of rescue (Fig. 3E),
262 suggesting that repeated genetic rescue may be a viable approach when there are no other
263 alternatives. Although much of the existing research on selecting source populations for genetic
264 rescue has focused on the issue of outbreeding depression (17, 18), we did not model this in
265 our simulations due to the strong assumptions that would be required on the genetic basis of

266 local adaptation (30). Moreover, an increasing number of studies have concluded that the risk
267 of outbreeding depression is probably quite low (18, 19, 28, 33), suggesting that it may be a less
268 important consideration than deleterious variation.

269

270 Taken together, our results paint a bleak picture for the future of the Isle Royale wolf
271 population, despite recent efforts to repopulate the island. Given the large ancestral wolf
272 population size and small carrying capacity of Isle Royale, extinction due to inbreeding
273 depression appears to be an inevitable outcome for any wolf population on the island (14, 15).
274 However, our results imply that the threat of extinction of the Isle Royale wolves and other
275 small and isolated populations might be substantially decreased if management strategies are
276 implemented that prioritize the minimization of deleterious variation. Rather than selecting
277 source populations with the goal of maximizing genetic diversity, future translocation initiatives
278 might instead target moderate-sized populations with a history of purging or screen large
279 populations for individuals with low levels of deleterious variation. Given the great expense of
280 most translocation programs, incorporating genomic tools represents a sound investment with
281 the potential to substantially postpone the need for future intervention.

282

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