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Fitness consequences and ancestry loss in the Apennine brown bear after a simulated genetic rescue intervention

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Abstract

Reduction in population size, with its predicted effects on population fitness, is the most alarming anthropogenic impact on endangered species. By introducing compatible individuals, genetic rescue (GR) is a promising but debated approach for reducing the genetic load unmasked by inbreeding and for restoring the fitness of declining populations. Although GR can improve genetic diversity and fitness, it can also produce loss of ancestry, hampering local adaptation, or replace with introduced variants the unique genetic pools evolved in endemic groups. We used forward genetic simulations based on empirical genomic data to assess fitness benefits and loss of ancestry risks of GR in the Apennine brown bear (*Ursus arctos marsicanus*). There are approximately 50 individuals of this isolated subspecies, and they have lower genetic diversity and higher inbreeding than other European brown bears, and GR has been suggested to reduce extinction risks. We compared 10 GR scenarios in which the number and genetic characteristics of migrants varied with a non-GR scenario of simple demographic increase due to nongenetic factors. The introduction of 5 individuals of higher fitness or lower levels of deleterious mutations than the target Apennine brown bear from a larger European brown bear population produced a rapid 10–20% increase in fitness in the subspecies and up to 22.4% loss of ancestry over 30 generations. Without a contemporary demographic increase, fitness started to decline again after a few generations. Doubling the population size without GR gradually increased fitness to a comparable level, but without losing ancestry, thus resulting in the best strategy for the Apennine brown bear conservation. Our results highlight the importance for management of endangered species of realistic forward simulations grounded in empirical whole-genome data.

KEYWORDS

endangered species, endemics, forward simulations, genomics, *Ursus arctos*

Consecuencias en la aptitud y pérdida de ascendencia del oso pardo de los Apeninos después de un rescate genético simulado

Resumen: La reducción del tamaño poblacional, con los previsible efectos sobre su aptitud, es el impacto antropogénico más alarmante sobre las especies amenazadas. Mediante la introducción de individuos compatibles, el rescate genético (RG) es una estrategia prometedora para reducir la carga genética revelada por la endogamia y restaurar la aptitud de las poblaciones en declive, aunque todavía se debate la eficiencia de esta. Aunque el RG puede mejorar la diversidad genética y la aptitud, también puede producir pérdida de ascendencia, lo que puede dificultar la adaptación local, o sustituir con variantes introducidas por los

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migrantes los acervos genéticos únicos que han evolucionado en grupos endémicos. En este trabajo realizamos simulaciones genéticas a futuro basadas en datos genómicos empíricos para evaluar los beneficios del RG en términos de aptitud y los riesgos de la pérdida de ascendencia en el oso pardo de los Apeninos (*Ursus arctos marsicanus*). Quedan aproximadamente 50 individuos de esta subespecie aislada que cuentan con una menor diversidad genética y un mayor nivel de endogamia comparado con otros osos pardos europeos y se ha sugerido que el RG podría reducir el riesgo de extinción de esta población. Comparamos 10 escenarios de RG en los que variaban el número y las características genéticas de los osos migrantes con un escenario sin RG con aumento demográfico causado por factores no genéticos. La introducción de 5 individuos procedentes de una población europea de oso pardo con mayor aptitud o niveles menores de mutaciones deletéreas que el oso pardo de los Apeninos produjo un rápido aumento de la aptitud del 10–20% en la subespecie y hasta un 22.4% de pérdida de ascendencia durante 30 generaciones. En las simulaciones sin un aumento demográfico, la aptitud empezó a disminuir de nuevo después de unas pocas generaciones. La duplicación del tamaño de la población sin RG aumentó gradualmente la aptitud hasta un nivel comparable al de algunos escenarios de RG, pero sin pérdida de ascendencia, por lo que parece ser la mejor estrategia para la conservación del oso pardo de los Apeninos. Nuestros resultados resaltan la importancia que tienen las simulaciones realistas a futuro basadas en datos empíricos del genoma completo para la gestión de especies amenazadas.

PALABRAS CLAVE

endemismo, especie en peligro, genómica, simulaciones a futuro, *Ursus arctos*

INTRODUCTION

The number of species and populations experiencing strong fragmentation due to anthropogenic impacts is dramatically increasing (Haddad et al., 2015; Steffen et al., 2007; Vitousek et al., 1997). The first and most prominent consequence of this is demographic (i.e., reduction in population size). With a primary focus on the demographic problem, conservation biologists initially supported more restrictive protection laws and suggested an increase in the number of individuals via translocation as a simple tool to stop population declines and compensate for the loss of individuals (Brown & Kodric-Brown, 1977; Hufbauer et al., 2015). Reductions in population size, however, may rapidly lead also to negative genetic effects that include inbreeding depression and accumulation of genetic load, phenotypically defined as the decrease in the average fitness of a population relative to the theoretically fittest genotypes (Crow, 1958). From a population genetics perspective, genetic load can be regarded as a statistic that summarizes the selection and dominance coefficients of all deleterious mutations (Bertorelle et al., 2022). Recent development of genomic tools has improved the ability to assess the genetic consequences of population decline and opened the opportunity to exploit genetic data to develop informed management practices for the protection of endangered species (Supple & Shapiro, 2018).

Although concerns about the lack of correlation between low genetic variation and reduced fitness in small populations have been raised (Aguilar et al., 2004; Benazzo et al., 2017; Fraser et al., 2014; Wood et al., 2016), wildlife corridors and animal translocation projects have proved to be effective tools for increasing genetic variation and boosting population growth

(Hogg et al., 2006; Madsen et al., 2020; Pimm et al., 2006). The genetic rescue (GR) approach, which shares some of the features of demographic rescue, prioritizes the genetic rather than demographic aspects of translocations. Even though the term *genetic rescue* has been around for over 50 years (Brown & Kodric-Brown, 1977), it has recently received renewed attention (Bell et al., 2019; Whiteley et al., 2015).

GR is at a mature theoretical phase, and mounting evidence suggests it can be more effective than demographic rescue, especially when resources allocated to conservation are scarce (Hufbauer et al., 2015; Weeks et al., 2017). Nevertheless, aspects of GR important to conservation (e.g., number of individuals to be translocated, their genetic characteristics, and their population of origin) remain unclear (Edmands, 2007; Frankham et al., 2011; Mills & Allendorf, 1996; Waller, 2015). On the one hand, those in favor of GR focus on the beneficial effects of heterosis and increased genetic diversity on fitness and growth rates, providing more efficient evolutionary responses to the changing environment. On the other hand, GR may reduce fitness and biodiversity through outbreeding depression and homogenization of divergent groups, the consequences of which would outweigh the potential benefits (Whiteley et al., 2015). Furthermore, even among GR supporters, there is a growing concern about the choice of the donor population, that is, whether to prioritize genetic diversity via translocations from large populations or from populations of intermediate size so as to reduce the risk of introducing large numbers of recessive deleterious variants (Bertorelle et al., 2022; Kyriazis et al., 2021; Ralls et al., 2020; Teixeira & Huber, 2021).

By introducing divergent individuals, GR may reduce the uniqueness of the endangered target population or species. The

loss of ancestral characteristics, observed in some populations following GR (Adams et al., 2011; Johnson et al., 2010; Miller et al., 2012), is a major drawback of this strategy, which aims to rescue, not replace, the endangered group. In this regard, immigrants' ancestry can rapidly increase in the rescued population, sometimes reaching levels that open questions of whether GR genetically replaces rather than rescues populations (e.g., immigrant ancestry is almost 60% in 2.5 generations of the gray wolf [*Canis lupus*] population in Isle Royale National park [Adams et al., 2011]). The extent of ancestry replacement is directly correlated to fitness improvement, which imposes finding a difficult trade-off between the 2 aspects when planning a GR. In addition, the magnitude and duration of GR beneficial effects remain unclear because most monitored rescues occurred only over the last 30 years, which is too short of a period in evolutionary and ecological times (e.g., Johnson et al., 2010; Madsen et al., 1999; Westemeier et al., 1998).

The remnant population of the Apennine brown bear or Marican bear (*Ursus arctos marsicanus*) survives in complete isolation in central Italy with about 50 individuals. Recent genomic data suggest that gene flow with other European brown bear populations ceased millennia ago, when major habitat fragmentation occurred due to forest clearing (Benazzo et al., 2017). Despite being legally protected since 1939, at which time there was immediate reduction of hunting and persecution, the population size has not varied (Ciucci & Boitani, 2008). The genomic variation of this group is indeed very low compared with other European brown bear populations (Benazzo et al., 2017), and recent population viability analyses indicate an extinction risk of 11–21% in 100 years (Gervasi & Ciucci, 2018). Whether this is sufficient evidence to support GR remains unclear. Recent studies describe the Apennine brown bear population as reproductively active, with no obvious signs of fitness reduction or loss of genetic variation at relevant immune system and olfactory gene loci (Benazzo et al., 2017). However, the situation could deteriorate in the near future, and GR could become a possible option. Therefore, we argue that predicting the consequences of GR under different parameters settings is good practice for the Apennine brown bear and other endangered species.

We used forward genomic simulations based on empirical genomic data to assess benefits and risks of GR in the small and isolated population of Apennine brown bear, which has much lower genetic variation than other brown bear populations. We modeled the demographic history of the Apennine brown bear and that of a hypothetical larger European population, which could represent a potential source of individuals for GR translocations. We aimed to predict and describe the dynamics of genetic load accumulation due to recessive deleterious mutations in this population and the positive (fitness increase) and negative (loss of ancestry) effects produced by GR. A GR intervention could be detrimental for the Apennine bear in terms of loss of local adaptations (e.g., loss of traits that facilitate coexistence with humans, such as small body size and reduced aggressiveness compared with other brown bears in Europe). The loss of ancestry could be regarded by local communities as a loss of an iconic natural resource and as an

economic loss because the bear is the most well-known attraction in the area. We predicted future bear populations based on different GR scenarios and no GR (i.e., assuming a purely demographic increase).

Local authorities are considering boosting the bear population by expanding the borders of the national park and by implementing additional conservation measures. These measures are expected to lead to an increase in the current population numbers of nearly 100% by 2050 (Ministero dell'Ambiente, 2011; WWF, 2019).

METHODS

We used computer simulations to predict the effects of translocating European brown bears from a relatively large population to the Apennine brown bear population. Our aim was to assess the consequences of translocations on fitness and ancestry of the Apennine bear population under different GR scenarios. Simulations were carried out in SLiM 3.4 and with a non-Wright–Fisher model (Haller & Messer, 2019).

Genetic model

A single chromosome was simulated to allow for time-effective modeling. Chromosome length and organization were defined in such a way to resemble a typical brown bear chromosome. Average chromosome, gene, and exon lengths were extracted from the grizzly bear (*Ursus arctos horribilis*) genome assembly (Taylor et al., 2018). The simulated chromosome was 62-Mb long, an average size considering that bears have 37 chromosomes and a genome size of 2.3 GB. The average length of genes, exons, introns, and intergenic regions was 40 kb, 300 bp, 15 kb, and 100 kb, respectively. We rescaled the fitness loss to the real genome size of 37 chromosomes based on the assumption that fitness effects act independently and multiplicatively. Under this common assumption, the loss of fitness, compared with an initial value, scaled to the number of chromosomes k following the relationship $1 - (1 - L_c)^k$, where L_c is the loss of fitness for a single chromosome. This relationship is almost linear when the load at each chromosome is low, and this was the case in our setting, as it is in some smaller scale simulations with genomes including 2–10 chromosomes (reported in Appendix S1).

In our scripts, only deleterious mutations were simulated because we assumed a minor effect of positive selection on locally adapted traits in the small Apennine population. We used SLiM's genealogical tree recording (Haller et al., 2019) to calculate statistics based on neutral mutations and used the genealogical trees to overlay neutral variants in a separate analysis with Python's pynslim and msprime (Haller & Messer, 2017). The mutation rate was set to 1.82×10^{-8} mutations per base pair per generation (Liu et al., 2014), and we considered a ratio of deleterious to neutral mutations of 2:1 for the exons and 1:4 for the introns (Harris & Nielsen, 2016). The recombination was assumed to be uniform across the simulated chromosome,

and we used a realistic rate of 10^{-8} crossing over events per base pair per generation (Dumont & Payseur, 2008; Lodish et al., 2008).

When a new mutation appears in a genome, its effect on individual fitness varies depending on the selection coefficient (s) and the dominance coefficient (h). The distribution of fitness effects (DFE) reflects the probability that a new mutation has a certain effect on an individual. It is therefore a crucial parameter that affects the overall genetic variability and the fitness of a population. However, because bear-specific DFE was not available, we used the human-estimated DFE (gamma distribution with mean $s = -0.043$ and shape parameter 0.23) from Eyre-Walker et al. (2006) as a reasonable measure of DFE in mammals. To further explore the effect of DFE on load dynamics, we repeated some simulations with a DFE with mean $s = -0.05$ and shape 0.5 as proposed in Kardos et al. (2021).

Together s and h mediate the effect of new mutations. Because most deleterious mutations are recessive or partially recessive (Fernández et al., 2004; García-Dorado & Caballero, 2000), we integrated the model with a gamma distribution of h with average 0.1 and shape parameter 1. The distributions of s and h used in the simulations are in Appendix S2.

Life-history model

The sexes were modeled under different reproductive behaviors. Although all individuals were modeled to begin reproducing from the fifth year of life, we considered a 1-year reproduction period in males and a 4-year interbirth period in females (Tosoni, Boitani, Gentile, et al., 2017; Tosoni, Boitani, Mastrantonio, et al., 2017). Age-dependent survival rates were set to 0.5 for the first year of life, 0.9 for the following 20 years, and 0.2 up to age of 30 years, when surviving individuals were forced by the model to die. These parameters were chosen based on estimates reported in previous studies on the Apennine brown bear (Tosoni, Boitani, Gentile, et al., 2017) and are broadly compatible with other European bear life-history traits. The probability of each individual being chosen as a parent for the next generation corresponded to fitness, which was reduced by the selection coefficient multiplied by dominance coefficient.

Demographic model

We initially simulated a single large population of 10,000 individuals over 350,000 years. This burn-in time allowed genetic variation to reach an equilibrium plateau (Figure 1). The number of individuals was controlled by the carrying capacity (i.e., the population size parameters fixed in the model), but small fluctuations were allowed and controlled by the SLiM algorithm with small changes to the average fitness. After the burn-in with the single large population, 2 isolated groups were generated to represent a generic large European population (the possible source of individuals for the GR of the Apennine brown bear) and the bottlenecked Apennine population. The European population had a stable population size of 2500 individuals, and

the Apennine brown bear population was stable at 2500 individuals for 2500 years, after which it declined to 50 (Figure 1). These values are in line with those estimated in Benazzo et al. (2017), who used whole-genome data. Therefore, the population at 5000 years from divergence represented the present population (time 0), and it was used as a starting point for analysis of the effects of different GR strategies. This part of the simulations was replicated 1000 times to take into account the mutational and genealogical stochasticity that may arise during the bottleneck.

Plausibility of simulation parameters

The plausibility of our genetic, life-history, and demographic models was supported by 3 lines of evidence. First, the average generation time that emerged in the simulations (calculated from the genealogical tree recorded in SLiM by averaging the distances between nodes in the trees with an ad hoc Python script based on *msprime* and *tskit* [Kelleher et al., 2016]) was 10.5 years. This value is similar to that estimated for this species (Gervasi & Ciucci, 2018). Second, the average number of surviving offspring per year in the simulated Apennine brown bear population (extracted directly from the SLiM output) was 7.4, which is compatible with the range observed in different years for the real population over the last 15 years (Tosoni, Boitani, Mastrantonio, et al., 2017). Third, the levels of genetic variation within and between the simulated populations (calculated with *tskit* [Kelleher et al., 2016]) were very similar to the values estimated from real genomic data. Genetic variation measured with the statistic θ ranges from 0.0015 to 0.0025 in individuals from different European populations and from 0.0005 to 0.0006 in different Apennine brown bear individuals (Benazzo et al., 2017), whereas average values in the simulated data were 0.0023 and 0.0006, respectively. The estimated divergence statistic (F_{ST}) computed between different European and the Apennine populations ranges in real data from 0.20 to 0.40, and the average value in the simulations was 0.31.

Alternative rescue strategies

We explored 4 main aspects of GR: number of introduced individuals, their sex, their genetic characteristic (diversity and fitness), and time of introduction. These variables were combined to obtain several scenarios (Table 1). For instance, in scenarios GR_5_Fit and GR_5_#mut, translocated individuals were selected based on their fitness (i.e., considering the effect of each deleterious mutation) or the total number of deleterious mutations, respectively. It is therefore assumed that a priori information on genetic load per individual is known so that identification of suitable candidates for GR can proceed. Each scenario was run for 300 years after GR translocations, and the key summary statistics were recorded at every step. A total of 12,000 independent simulations were run (1000 for each scenario).

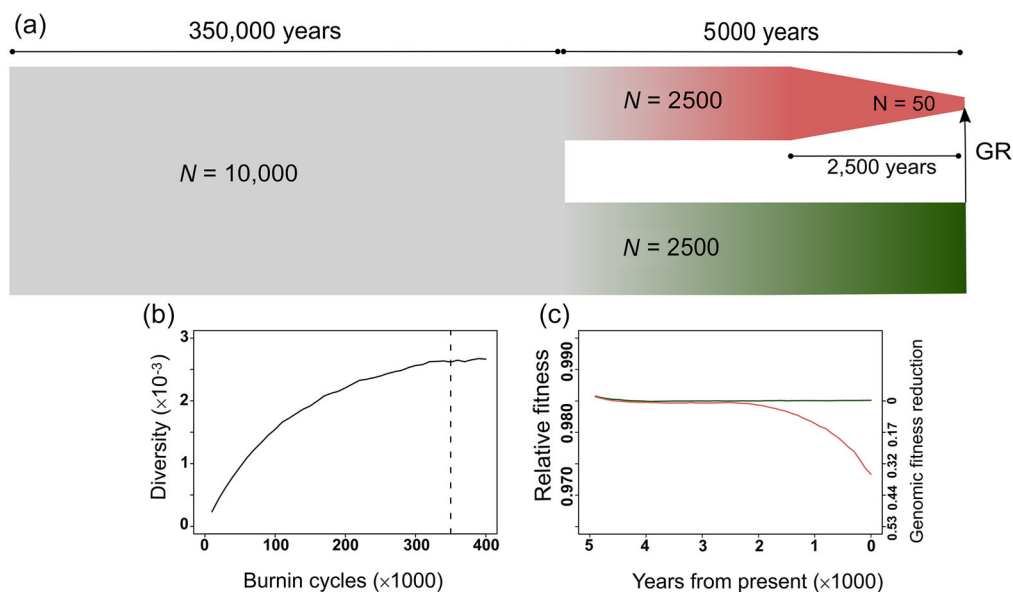


FIGURE 1 (a) Demographic model of brown bear populations used in the simulations (N , population size; red branch, Apennine population; green branch, larger European population; GR, genetic rescue), (b) genetic diversity (heterozygosity) monitored during the burn-in for the ancestral population (dashed line, point at which equilibrium was reached and burn-in was considered complete), and (c) variation in fitness in the 2 simulated populations after the split (red, simulated Apennine population; green, simulated European population; left axis, fitness at 1 chromosome [our simulation setting]; right axis, projected reduction of fitness at 37 chromosomes in the Apennine bear population compared with fitness of the European bear population).

TABLE 1 Description of 10 scenarios of genetic rescue (GR) and 2 scenarios without genetic rescue (NoGR) simulated for the Apennine brown bear.*

Scenario code	Description
NoGR	Apennine population stable at 50 individuals and no GR performed (control scenario)
GR_5	Five random individuals introduced at time 0
GR_10	Ten random individuals introduced at time 0
GR_5+5(10)	Five random individuals introduced at time 0 and 5 additional individuals introduced after 10 years
GR_5+5(40)	Five random individuals introduced at time 0 and 5 additional individuals introduced after 40 years
GR_5m	Five male individuals introduced at time 0
GR_5f	Five female individuals introduced at time 0
GR_5_Fit	Five fittest individuals introduced at time 0
GR_5_#mut	Five individuals with the lowest number of deleterious mutations (lowest load) introduced at time 0
GR_25	Twenty-five random individuals introduced at time 0 (extreme GR used for comparison)
NoGR_exp	Apennine population allowed to grow to 100 individuals and no GR performed
GR_5Fit_exp	Five fittest individuals introduced at time 0 and Apennine population is allowed to grow to 100 individuals

*Excluding the last 2 scenarios, the carrying capacity of the Apennine population was fixed at 50 individuals in the model.

Monitoring effects of different scenarios

Single individuals were monitored before and after time 0 (i.e., present time) with fitness and ancestry. The fitness value of any mutation with selection coefficient s and dominance coefficient b was calculated as $(1 - s)$ for a homozygous locus and $(1 - bs)$ for a heterozygous locus, and the fitness of an individual was then calculated multiplicatively across loci. Finally, the overall population fitness was computed as an average across all individuals.

Ancestry is the proportion of Apennine genomes retained after GR, and it was monitored using the method of Harris et al. (2019) and Harris and Nielsen (2016). We added 10,000 neutral markers (European markers) to each haploid genome of the introduced individuals, resulting in a density of approximately one marker every 6200 bp. Following introduction (GR), these markers were expected to spread into the Apennine population as entire chromosomes in the first generation and as genome fragments in the following generations due to recombination. By counting the number of these markers in each individual's

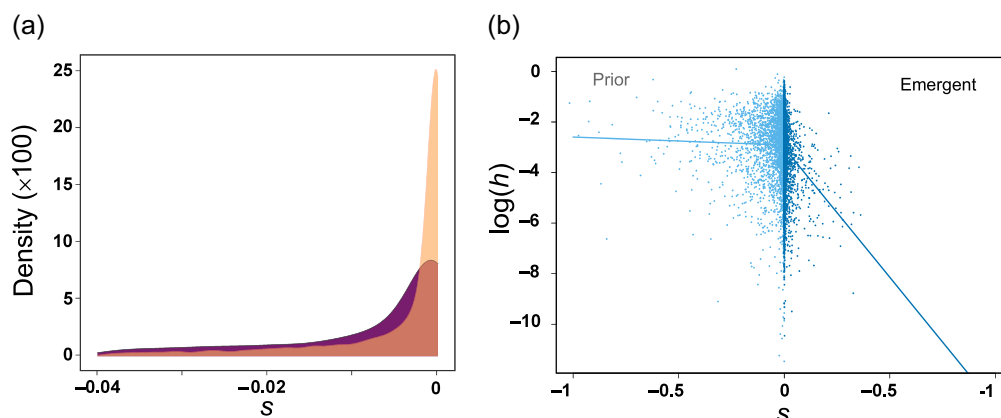


FIGURE 2 Statistics after the burn-in simulations for the ancestral population of Apennine brown bear: (a) distribution of selection coefficients (s) based on the human distribution of fitness effects (purple) and emergent distribution of selection coefficients after the filtering of selection (orange) and (b) relationship between selection coefficient and the log of dominance coefficient (light blue, result from s and h values from the prior distribution; dark blue, emergent values for h and s ; x-axis is mirrored on the right side of the plot; lines, exponential model that best fit the data).

genome, we were able to calculate the genomic proportion of introgressed markers. Its complement to unity is the Apennine residual ancestry. For example, at a certain point after GR, an individual might have 5000 European markers. The Marsican ancestry is then estimated as $1 - 5000/20,000 = 0.75$.

RESULTS

Simulation cycles before translocation

After 350,000 burn-in cycles, the average diversity of the burn-in population plateaued at approximately 0.0026 (Figure 1b). At this point, the number of deleterious mutations in the system was 8611, of which 232 were fixed across the ~10,000 individuals.

The DFE after the burn-in shifted toward neutral values (smaller selection coefficients) when compared with the prior distribution (Figure 2a). The average s (considering only mutations with minimum allele frequency of 0.5%) was -0.0021 . In contrast, the average h stabilized at approximately 0.09, closely resembling the value used in the prior distribution (0.1). The negative correlation between s and h , absent when the values of these variables were generated, was fitted by an exponential relationship after the burn-in (Figure 2b). This effect is related to the so-called Haldane's sieve (Haldane, 1924; Turner, 1981). We found significant negative relationship between s and the logarithm of h ($p < 0.001$) (Figure 2b).

The bottleneck starting 2500 years before present affected the fitness of the Apennine population. The relative fitness reduction estimated in the single-chromosome simulations was 1.2% (average across 1000 simulations), corresponding to an expected loss of 36% when scaled to 37 chromosomes (Figure 1c). At the end of the bottleneck, the individual diversity calculated at all sites (deleterious and overlaid neutral) and averaged over 1000 simulations were 0.0023 (mean and 10–90% quantiles coincided) and 0.0006 (0.0005 and 0.0008 for 10% and

90% quantiles, respectively) for the European and the Apennine brown bears, respectively. Average F_{ST} was 0.3117 (0.2924 and 0.3328 for 10% and 90% quantiles, respectively). Individual diversity ranged from 0.0015 to 0.0025 in the European brown bear and from 0.0005 to 0.0006 in the Apennine brown bear, and their F_{ST} values ranged from 0.20 to 0.40, depending on the European population considered.

On average, each European individual carried 587.1 deleterious mutations, distributed in 242.9 and 172.1 heterozygous and homozygous sites, respectively. Each Apennine individual had on average 499.2 deleterious mutations, distributed in 72.2 and 213.5 heterozygous and homozygous sites, respectively.

Simulation cycles after the translocation

As expected, the introduction of European bears improved average fitness and reduced ancestry (fraction of ancestral genomes) in the Apennine group (Figure 3). Complete results produced under the different rescue strategies we simulated are in Appendices S3–S29.

Modification of DFE mean and shape for the models GR_5Fit and GR_5Fit_exp produced only minor changes in the load dynamics that did not affect the general patterns (Appendix S29). We report the most relevant patterns only.

Effects of increasing number of introduced individuals

In approximately the first 20 years, the fitness increase in the Apennine population was directly related to the number of individuals introduced (Figure 3a). After GR, fitness increased by 10%, 20%, and 31% when 5, 10, or 25 individuals were introduced, respectively. After this short period, fitness slowly returned to starting values. After 200 years, the fitness gain was more than halved and more similar across scenarios with

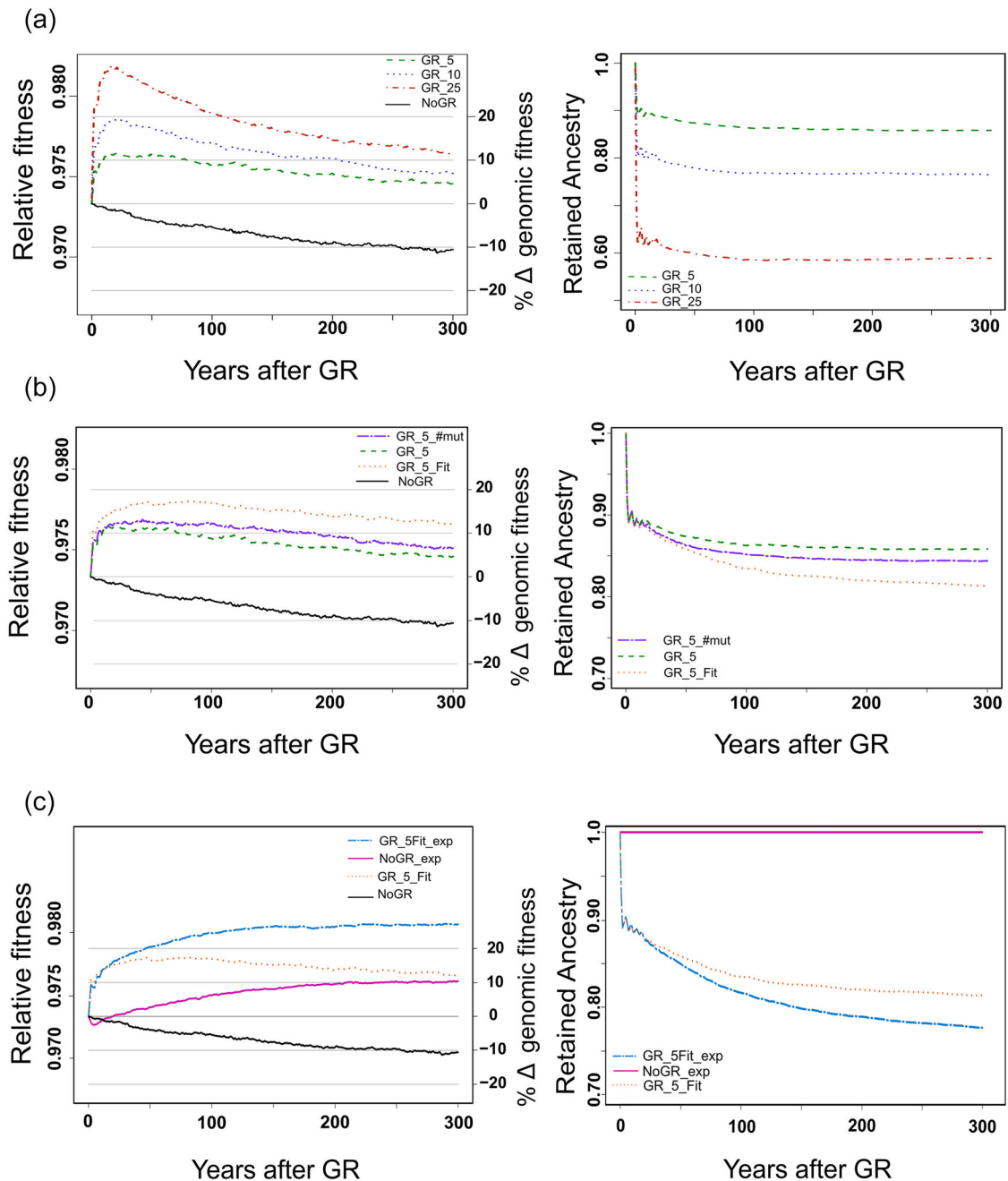


FIGURE 3 Effects of introducing (a) more individuals and (b) genetically selected individuals to a population of Apennine brown bears and of (c) allowing the population to grow on fitness and ancestry (solid line, trajectory of the control scenario [i.e., NoGR]; left column, y-axis fitness at 1 chromosome [our simulation setting]; right axis, projected reduction of fitness at 37 chromosomes in the Apennine population compared with fitness of the same population at time 0; model scenarios defined in Table 1).

different numbers of introduced individuals (4.7%, 6.9%, and 11.6% for GR_5, GR_10, and GR_25, respectively). When we introduced 10 individuals and allowed them to reproduce for one generation only and then removed them from the population, fitness gain was slightly less than that in GR_5 (Appendix S27). For Apennine bears, ancestry instantaneously decreased by around 10%, 20%, and 37% for GR_5, GR_10, and GR_25,

respectively, and then reached a plateau after around 100 years, when ancestry loss was 13.7%, 23.1%, and 41.4% for GR_5, GR_10, and GR_25, respectively. The ancestry proportion when 10 individuals were introduced but removed soon after the first reproduction, after the initial decline due to fraction of European individuals, stabilized to the value observed under GR_5 (Appendix S28).

Effect of selecting individuals

The translocation of the 5 fittest individuals (Figure 3b) resulted in a higher increase in fitness after 50 years compared with GR_5 and a similar increase in fitness under GR_10. This strategy allowed maintenance of an Apennine ancestry level similar to that observed under the scenario with 5 introduced individuals (Figure 3a,b). For GR_5_Fit, the loss of Apennine ancestry was 10% after the first few years (as for GR_5), but then its plateau was less evident and ancestry loss increased more steadily, reaching 15.4% after 100 years and 18.6% after 300 years. The loss of fitness gain in the first years after GR was less evident than that in subsequent years. After 300 years, fitness was still 12.3% higher than before GR (i.e., higher than for GR_25 at the same period). The selection of 5 individuals based on their number of deleterious mutations (GR_5_#mut) provided an improvement in Apennine fitness between those of model GR_5 and model GR_5_Fit, and so did the ancestry loss pattern (Figure 3b).

Effect of demographic increase

The results of the scenarios in which the Apennine population was allowed to grow to 100 individuals without GR (NoGR_exp) showed that fitness slowly but steadily increased to levels very similar to those of GR_5_Fit, but with the advantage of keeping the Apennine ancestry intact (Figure 3c). After 300 years, fitness improved by approximately 10% under both scenarios. When demographic increase and GR_5_Fit were combined, the gain in fitness was at its maximum, and the trend increased continuously, reaching a gain of 27.2% after 300 years. In this case, the Apennine residual ancestry diminished throughout the period and reached 22.4% of ancestry loss after 300 years, which is similar to the loss recorded for GR_10 after the same period.

The other scenarios did not differ significantly in terms of fitness change or ancestry loss. The sex of the introduced individuals had no effect on these 2 aspects (i.e., introducing 5 males, 5 females, or 5 mixed individuals [Appendices S5, S6, & S13–S16]) and neither did introducing 10 individuals at once or in 2 groups separated by 10 or 40 years (Appendices S7–S12).

DISCUSSION

This study was motivated by a simple but crucial question: What are the likely consequences in terms of fitness recovery and ancestry loss of a GR intervention applied to the small, isolated, and highly inbred Apennine brown bear population? The question is simple but the answer is not because it depends on several genetic and nongenetic factors, including future habitat changes and the expected results of planned improvements in monitoring and protection activities. We argue that even if the situation of the last Italian native population of brown bears is precarious, it can still be managed without the need to resort to individual translocations from a different genetic pool. However, we

also acknowledge that the situation could deteriorate in the near future. In this context, our simulation-based approach to analysis of the likely genetic effects of GR should be considered a useful exercise to aid future management for the conservation of the Apennine brown bear and an example that can guide similar studies of other endangered species.

In general, our simulations showed that introducing a few bears from a different and more variable population increased the average fitness of the Apennine population by reducing the effects of deleterious mutations. At the same time, however, this intervention brought significant changes to the genetic pool of the native population. These effects were expected and magnified when more individuals were introduced. The simulations, however, provided more specific and informative details about the temporal dynamic of the GR effects under different GR regimes and facilitated the comparison between strategies before their future implementation.

Average fitness increased significantly in <50 years after GR, but without a demographic expansion, it gradually declined to the initial values. The increase was rapid because many deleterious mutations (also at intermediate frequencies due to large drift effects) were masked by introduced alleles, and the decline, related to reestablishment of the inbreeding consequences, was slower. However, after 200 years of drift and inbreeding at the same small population size, approximately one half of the initial fitness rise was lost. The peak of fitness depends on the number of introduced individuals and therefore on the number of allochthonous genetic variants. Fitness dynamics were very similar with 5 or 10 introduced animals and did not vary in our setting with sex ratio or when individuals were introduced simultaneously or at different times. In sum, the benefit of GR in terms of reducing genetic load and increasing genetic variation appeared short lived. It is also possible that the introduced individuals, sampled in the highly variable bear population where many deleterious mutations are present at low frequencies, contributed significantly to the fitness decline when increasing in frequency in the rescued population. This point is clearly linked to the recent debate about the choice of the best source population for GR (Kyriazis et al., 2021), and it should be simulated in future studies considering the negative consequences of introducing deleterious variants and the positive effects related to high levels of standing variation in terms of increased adaptability to changing environments.

Our results validated the importance of favoring the increase of the population size to recover some of the fitness lost during the bottleneck. Even without GR, the inbreeding effects were mitigated when the population expanded from 50 to 100 individuals, and selection more efficiently reduced random fluctuations of deleterious variants, producing a slow but constant increase of the average fitness. When population growth was combined with a GR intervention, the fitness increase was rapid and constant (until a plateau was reached), making this strategy the most efficient in our study.

Careful consideration should be given to the origin of each individual before selecting candidates for GR translocation. Classical recommendations support the use of individuals from highly variable and ecologically similar populations to maximize

evolutionary potential and avoid inbreeding and outbreeding depression (Finger et al., 2011; Frankham, 2015; Pickup et al., 2013; Ralls et al., 2020), and these criteria can be applied to populations but also, if data are available, to individual candidates. However, negative impacts are also predicted when individuals from highly variable populations are introduced. The idea that the most appropriate individuals for GR are those coming from environments similar to those of the rescued population is also questionable, especially in a time of rapid climate change, when the adaptational lag can be strong enough such that individuals growing under certain conditions might not be the best adapted to current conditions (Browne et al., 2019). Here, we showed that individuals carrying the smallest genetic load (model where individuals were selected according to fitness), or simply with the fewest number of deleterious mutations, should be prioritized to increase efficiency of Apennine brown bear rescue. The selection based on genetic load provided the best results in terms of fitness gain, but this approach, should it be applied in the real world, would require accurate information on the genome of the species involved, including reliable predictions of mutation effects and dominance. Because the theory and the bioinformatic approaches to address these topics are evolving rapidly (Bertorelle et al., 2022; Robinson et al., 2018, 2019), an approach based directly on the estimated load of individuals will likely be feasible soon. To date, the most accessible source of information is the number of these mutations in the genome. A plethora of tools have been developed for predicting whether a mutation can be deleterious based on its annotation or evolutionary conservation, and these predictions are becoming increasingly reliable (Bertorelle et al., 2022; Huber et al., 2020; Lopes et al., 2012).

The loss of ancestry followed a rather simple dynamic after GR. It dropped rapidly to the value expected from the fraction of introduced individuals in the rescued population and then lessened slightly over the next few hundred years. The second decline occurred because the descendants of introduced individuals, in the first generations and before mixing with the native individuals (which is a relatively rapid process; see Kalinowski & Powell, 2015), still benefitted from a fitness advantage that favored the spread of introduced genomic fragments. This hypothesis is supported by the fact that when the introduced individuals are selected as the fittest or those with the smallest number of deleterious mutations, the secondary ancestry decline is more evident. When homogenization is complete, the ancestry level stabilizes. The plateau of ancestry is slightly lower, in case of demographic increase, probably because drift effects on the introduced variants are reduced. These results, as well as those regarding the fitness dynamic, are based on 1000 replicates of a stochastic process. In our simulation, for example, the average retained ancestry 100 years after GR with 10 individuals was ~ 0.77 (Figure 3), but there was still a 20% chance that this ancestry would be < 0.7 (Figure 4). In other words, the targets for the retained ancestry should be probabilistically defined, and the best GR should be identified accordingly. Fragmentation of the Apennine chromosome after GR should also be taken into account when evaluating the loss of ancestry. Even a small number of introduced individuals can produce after some

generations of random mating and recombination an admixed population in which most individuals have mosaic genomes and have lost long and continuous fragments of Apennine origin, making recovery of ancestral genomes impossible.

Model limitations

We used a set of realistic parameters to simulate the genomes, demography, history, and life history of the biological systems we analyzed. This is very uncommon in simulation studies, where several aspects of genetics (e.g., when using Vortex [Lacy & Pollak, 2021]) or life history (e.g., when using coalescent based simulations [Excoffier et al., 2021]) are often neglected. For our models, data from many studies on the brown bear and the highly flexible SLiM (Haller & Messer, 2019) simulator were available. Nevertheless, there are limitations to our analyses, shared with other studies with similar approaches [Matz et al., 2018; Robinson et al., 2019]. First, the reduced genetic model we used (one chromosome instead of 37 with independent loci) is computationally tractable, but the impact of gene interactions at the genome level was not considered. Second, fitness in the rescued populations was relative (in comparison with a larger population or before and after an intervention) and did not include positively selected mutations and current or future adaptations to changing environment, and it was decoupled from the demography of the populations (i.e., extinctions are not analyzed).

Population size was constrained by a fixed carrying capacity and was not affected by the total population fitness. In this so-called soft selection model (Haller & Messer, 2019; Wallace, 1975), higher success for some individuals will mean lower success for others, keeping population size stable. Because Apennine bears have lived in isolation at a small population size for a few thousand years without evidence of decline (at least in the last 50 years), understanding the relative effects of different translocation strategies on genetic load reduction in a few generations is very likely unaffected by the use of soft or hard selection models or by considering or not adaptive mutations. We believe these factors may have had an impact on the absolute values of the statistics we evaluated, but the outcome of cross-scenario comparisons and the overall conclusions were not affected. The complexity of a biological system can be virtually infinite, and all the efforts to theoretically identify the general patterns of genetic load accumulation and purging (Byers & Waller, 1999; Hedrick & Garcia-Dorado, 2016; López-Cortegano et al., 2021) in highly simplified models are hindered by factors that can be simultaneously considered (Bertorelle et al., 2022). The simulation model we developed specifically for the Apennine brown bear is an efficient representation of several realistic aspects of the system, and it is useful to refine the general conclusions suggested by theoretical analyses even without considering all the factors. Our simulations captured the main characteristics of the genetic load accumulation in the Apennine brown bear and can hence be used to support development of new and more evidence-based protection strategies for this population.

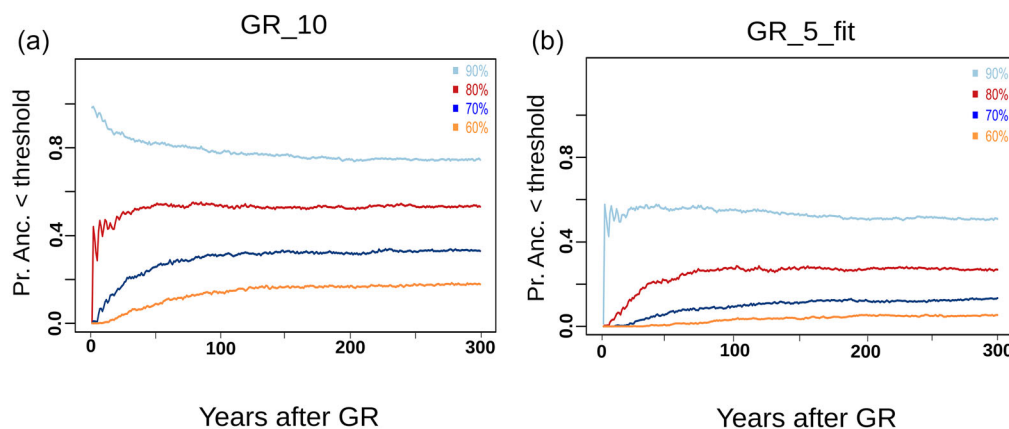


FIGURE 4 The probability over time of a 90%, 80%, 70%, and 60% reduction in Apennine bear native ancestry in the GR₁₀ and GR_{5_Fit} scenarios (defined in Table 1).

GR for the Apennine brown bear

Because the Apennine brown bear likely developed some level of local adaptation (Colangelo et al., 2012) and has reduced aggressiveness compared with other brown bears (Benazzo et al., 2017; Thompson, 2018), it has retained genetic variation at important genomic regions (Benazzo et al., 2017) and does not have dramatic signatures of reduced fitness at the phenotypic level (Colangelo et al., 2012). Thus, GR is not immediately needed. Our results are, however, highly and practically informative. First, if new evidence were to suggest an increased risk of extinction, GR could be implemented with as few as 5 individuals. To further rapid increase in the average fitness of the population, it would be best to select individuals, after genome sequencing, with as few deleterious variants as possible. Favoring a subsequent demographic increase would be highly recommended to prevent fitness from declining again in the long term.

Second, a demographic expansion to 100 animals without GR, a desired objective for the Apennine brown bear (WWF, 2019) but apparently difficult to obtain, could also further an increase in average fitness, but this increase is predicted to be very slow. It cannot be ignored that, if urgently needed, the introduction of few animals and the consequent fitness increase could drive demographic expansion (Hedrick & Fredrickson, 2010; Madsen et al., 2020), magnifying the fitness effects. Third, if locally selected variants in the Apennine brown bear play a minor role, the loss of Apennine ancestry is mainly guided by the initial step of the introduction with only a limited effect related to the subsequent spread of genomic regions from the introduced animals. This result facilitates the planning of a future GR intervention after the identification of the target in terms of tolerable loss of ancestry, which should, however, be defined probabilistically considering the stochastic component of the process and evaluated considering the possible loss of locally adapted variants and the level of acceptance of local communities to introductions of allochthonous individuals and divergent phenotypes. An integrated, scaled approach to

measuring the biological, social, political, and economic advantages and disadvantages of a GR strategy will always require a multidisciplinary team of experts (IUCN/SSC, 2013).

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SUPPORTING INFORMATION

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