

Comment on “Individual heterozygosity predicts translocation success in threatened desert tortoises”

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Abstract

Scott et al. (Reports, 27 November 2020, p. 1086) bring much-needed attention to species conservation by demonstrating heterozygote-superiority among translocated tortoises. However, we believe that their recommended heterozygosity decision-rule risks taking conservation genomics backwards. We argue that their advice could misguide conservation management aimed at establishing viable populations, and that it can be improved by also assessing the genetic load.

Scott et al. (1) utilize a fascinating conservation programme in the threatened Mojave desert tortoise (*Gopherus agassizii*) that took place between 1996-2014, during which almost 10,000 individuals were translocated and released in the wild to roam freely. Some of the individuals are still alive today (~4%), and Scott et al. (1) show that these surviving tortoises possess significantly higher genome-wide heterozygosity (~23%) than those that died. In contrast, genetically inferred translocation distance did not explain individual survival success. Scott et al. (1) concluded that prioritizing heterozygosity could be an optimal strategy for reintroductions. We argue that (i) this recommendation oversimplifies conservation genomics by ignoring critical data, (ii) the advice would misguide conservation actions that aim to establish viable populations, and (iii) translocation actions can be significantly improved by also assessing the genetic load of individuals.

The advent of molecular markers was a milestone in conservation, enabling assessment of inbreeding effects in free-living organisms. Studies in the past 50 years have highlighted the importance of conserving genetic variation, and positive correlations between heterozygosity and fitness are found across a variety of taxa (2, 3). Scott et al. (1) contribute rare experimental evidence, demonstrating the importance of heterozygosity for survival. We are continuously refining our understanding of genetic erosion in declining populations, and how viability of such populations is determined by both genetic variation (heterozygosity) and exposure of

genetic loads (mainly, expression of recessive deleterious variants in homozygous state) (4). Recent studies shed light on how the genetic load affects populations with different demographic history, and extinction risk as assessed on the IUCN Red List (5, 6). Genomic data can be used to delineate recent and historic inbreeding (7), and a new generation of software utilizes genome data to infer past population demographics (8). The next challenge in conservation is to understand how fitness in the wild is determined by specific genetic variants, going beyond assessing heterozygosity (9). Comparative genomic techniques such as genomic evolutionary rate profiling (GERP) and combined annotation-dependent depletion (CADD) are employed to quantify the genetic load at the molecular level (9, 10). This is a critical step-change, because although maintaining genetic variation is an important goal in conservation, for many species we are losing the battle. Most vertebrate species are in severe decline (11), and the small captive populations in zoos and aquaria are prone to genetic drift and inbreeding, resulting in detrimental fitness effects due to the expression of genetic load.

Scott et al. (1) showed that the mean heterozygosity of the surviving tortoises was 23.09% greater compared to those that died, and they suggest an optimal reintroduction strategy for promoting survival by prioritizing translocation of the most genomically variable individuals. We used computer simulations in a metapopulation framework to examine the generality and consequences of this advice in future generations. Our simulations show that translocating individuals with the highest genetic diversity ('Diversity Regime'), i.e., the advice proposed by Scott et al. (1), indeed increases the fitness and nucleotide variation in the translocated cohort compared to random sampling ('Random Regime') (Fig. 1A,B). However, these benefits are transient as the genetic load is highest in the Diversity Regime (Fig. 1A-C). By selecting individuals with lowest genetic load ('Load Regime') fitness is initially maximized (Fig. 1A), and importantly, this fitness-gain is sustained over generations because deleterious mutations have been purged (Fig. 1C). However, both the Diversity Regime and Load Regime bias the representation of some subpopulations and genetic lineages (Fig. 1D), and this unequal genetic representation during translocation increases genetic drift in subsequent generations compared to the Random Regime, reducing genetic diversity (Fig. 1B). Interestingly, however, by applying a translocation regime combining individuals with the lowest genetic load and the highest genetic uniqueness ('Load+Diversity Regime'), it is possible to strike a balance and both reduce the genetic load, as well as increase fitness and genetic variation (Fig. 1A-C). Our simulations illustrate that if the long-term viability of populations is the conservation goal, prioritizing only heterozygosity is not advisable.

Scott et al.'s (1) recommendation of prioritizing heterozygosity in conservation also neglects that ecological heterogeneities can drive fitness-associated adaptations to local conditions. In such circumstances, translocations from closely located donor sources should be considered when these populations are not too genetically depauperate. The vivid conservation debate around the Florida panther (*Puma c. concolor/coryi*) (12) illustrates the complexity of this point.

Scott et al. (1) state to have tested, as they phrase it, the “conventional wisdom” in conservation of exclusively translocating individuals from nearby populations. However, the translocation distances used are likely of low accuracy as the tortoises’ place of origin was inferred by blunt genetic assignment methods, adding substantial noise to the data. Moreover, the relative importance of translocation distance and heterozygosity in conservation is a serious issue only in species exhibiting local adaptations, which remains unknown in desert tortoises. Even without local adaptations, our simulations show that translocating the most diverse individuals increases genetic drift and genomic erosion in the long term.

To conclude, Scott et al. (1) demonstrate significant heterozygote-superiority in the Mojave desert tortoise, thereby providing a tantalizingly simple decision-rule for conservation managers. Our simulations suggest that artificially translocating heterozygous individuals only results in transient fitness benefits, as genetic erosion accelerates in subsequent generations. A regime minimizing genetic load can increase fitness even further, but it too suffers from increased genetic drift. We propose a balanced approach that minimizes genetic load whilst retaining variation, and our simulations suggest this might present the best of both worlds. However, real life is substantially more complex than simulations, and we do not wish to erect unsurmountable barriers for conservation actions. Indeed, the cost of inaction often outweighs the costs of action (13). Nevertheless, we argue that recent advances in genomics and bioinformatics allow for better assessments of population viability (9, 10), and that we need to do more than simply conserving genetic variation by also minimising the genetic load. How to exactly balance these multiple genomic considerations in conservation management will be an arena for healthy debate.

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ACKNOWLEDGEMENTS

This project received funding from the Swedish Research Council (consolidator grant 621-2016-689 to BH), the European Union's Horizon 2020 research and innovation programme (Marie Skłodowska-Curie grant 840519 to HEM), the Earth and Life Systems Alliance (ELSA) of the Norwich Research Park, Norwich, UK (to CvO).

Fig. 1 Individual based simulations show that alternative translocation regimes can have differential effects on genetic variation and fitness. Random Regime (yellow): random sampling of individuals from a metapopulation consisting of four subpopulations; Diversity Regime (blue): artificial selection of individuals with the highest 20% of nucleotide diversity; Load Regime (red): artificial selection of individuals with the lowest 20% of genetic load. Here, we define the genetic load of an individual (L_{ind}) as the sum of the selection coefficients of all deleterious mutations that are in homozygous condition, plus the sum of selection coefficients multiplied by their dominance coefficients of all deleterious mutations that are in heterozygous condition: $L_{ind} = \sum_i s_i + \sum_j h_j s_j$. Load+Diversity Regime (orange): two-step approach in which individuals with the lowest 30% of genetic load are selected first, followed by the selection of the top 50% of individuals with most rare neutral genetic variation. To this end, an individual's genetic uniqueness score (U_{ind}) is calculated as $U_{ind} = \sum_i (1 - q_i)$, where q_i is the allelic frequency at locus i . This two-step approach is done per subpopulation to ensure all subpopulations contribute, thereby capturing more variation. **(A)** The Diversity Regime, the advice proposed by Scott et al. (1), increases the fitness of translocated individuals compared to the Random Regime, but this fitness benefit is transient and disappears in the subsequent generations. The Load Regime and Load+Diversity Regime result in a higher fitness sustained over generations. **(B)** Nucleotide diversity is highest in the Diversity Regime directly after translocation, but it drops relative to the Random Regime in subsequent generations. The Load Regime shows the lowest diversity, whereas the Load+Diversity Regime shows the highest diversity in the post-translocation generations. **(C)** The part of the load that is in heterozygous condition ($\sum_j h_j s_j$), the load that causes inbreeding depression in future generations, is the lowest in the Load Regime and Load+Diversity Regime, and highest in the Diversity Regime. **(D)** The Random Regime contributes individuals from all populations, and it samples the highest percentage of lineages. The Diversity Regime and the Load Regime both reduce the population representation and the percentage of contributing lineages in the translocated cohort. This increases the level of genetic drift, reducing the genome-wide diversity in subsequent generations. The Load+Diversity Regime effectively removes genetic load and retains nucleotide diversity by sampling individuals with rare variation and low load across all populations. We simulated 100 replicates per regime of a 1Mb chromosome with a mutation rate $\mu = 1e-7$ and recombination rate $\rho = 1e-8$. The ratio of deleterious to neutral mutations is 0.2:1. During the burn-in, four populations with an $N_e = 500$ accumulated mutations for 5000 generations. Next, individuals were selected at random or based on their nucleotide diversity and/or genetic load, and these were then translocated to an admixed population where they mated randomly. The plots depict the distribution of mean values per replicate. Simulations were performed with SLiM3 (14). The code of these simulations and results for other scenarios can be found in ref. (15).

