Genetic rescue of small populations in the presence of deleterious variation

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Abstract
Genetic rescue using external breeders or cryo-preserved semen is a reasonable strategy to restore diversity in small breeds. It is also known from natural populations that animals from large populations can convey substantial genetic load when they migrate into small, highly inbred populations, which may compromise the recipient population. We used stochastic simulation to evaluate different scenarios of genetic rescue of two conservation stocks of different sizes and found that introgression of males from larger stocks had a more detrimental effect on the viability of offspring than from smaller stocks, even though both strategies partially restored diversity. Using samples from gene-banks had a comparably negative effect on offspring viability, if the population was substantially larger at the time of cryo-conservation. Our results suggest that careful assessment of genetic load and its consequences is required whenever external animals should be transferred into endangered stocks. As larger breeds have higher genetic load due to less efficient purging, animals from small breeds seem beneficial for genetic rescue.

Introduction
The conservation of genetic diversity is of major concern in many livestock species as commercially less preferred breeds are often decreasing in size, leading to a high degree of inbreeding. It is believed, that such breeds will consequently suffer from inbreeding depression (Charlesworth & Willis, 2009) and might eventually become inviable (Soule, 1987). Deleterious variants seem to play an important role, even though the actual mechanisms are under debate (Charlesworth & Willis, 2009) and their distribution and frequency in livestock might be strongly dependent on population characteristics, demography and breeding practices (Bosse et al., 2019). At the same time, strong recessive deleterious variations are subject to constant selective pressure called purging, which is even more efficient under high levels of inbreeding, leading to viable, though highly inbred, populations (Robinson et al., 2018).

Conservation schemes based either on introgression of animals from related, but numerically larger populations (Kyriazis et al., 2021) or insemination with cryo-preserved semen from gene banks (FAO, 2012) are considered as a reasonable strategy to restore genetic variability and counter inbreeding depression. Albeit, positive effects generally outweigh the negative (Frankham, 2015), outbreeding depression is a concern. Observations from an island grey wolf population, where a male from a much larger population entered a highly inbred insular stock, putatively introducing strongly deleterious variants, resulted in the extinction of the respective population (Kyriazis et al., 2021). This implicates that the aforementioned strategies might not be without risk also in livestock. The aim of this study is to evaluate the effect of animal migration from larger populations and a cryo-conserve into smaller stocks in a livestock setting with specific focus on strong deleterious variations.

Materials & Methods

We used stochastic simulation with the R-package MoBPS (Pook et al., 2020) and started with an initial pig population of 1000 pigs with an equal sex ratio. Variants and maps were taken from Ensembl (Howe et al., 2021). First, a quantitative trait with 1,000 purely additive QTLs was simulated to mimic a performance trait like 6-month weight. Second, 1,000 recessively lethal deleterious variants were randomly placed across the genome. Frequencies of the deleterious variants were sampled from a normal distribution with a mean of 1 % and a standard deviation of 0.5 %, while setting values below zero to zero. This results in a population with about 88% of all meiosis in the founder population leading to viable offspring.

The simulation was divided into three main stages. *S1*: Initial 15 generations in a breeding stock of equal sex ratio to generate a viable start population followed by *S2*: deriving four lines, *Large (L), Medium (M), Endangered (E)* and *Critical (C)* and breeding with constant to drastically decreasing size and a sex ratio of 1:2 for 20 generations. Animal numbers were cascaded down sequentially (**Table 1**). Mild selection for 6-month weight was applied to all stocks in *S1* and *S2*.

In *S3*, genetic rescue of the *Endangered* and *Critical* stock was modelled in scenarios by mating always two males from other stocks or a cryo-conserve, taken from the stock to be rescued ten generations ago, with all females, with the exception of no mating between *M* and *C*. After genetic rescue, selection was switched to random mating with constant stock size and sex ratio of 1:2. Proportions of purged deleterious variants, average deleterious allele content per animal, the proportion of offspring being homozygous for at least one lethal allele, and inbreeding rates (calculated based on share of the genome in Identity-by-Descent) were tracked through all generations. All simulations were repeated 500 times. The interested reader is referred to https://github.com/tpook92/MoBPS/ for the R-Script.

Table 1. Simulated stock sizes in *S2*.

| Stock	Generation	17	18-21	22-26	27-36
Large		1,000	1,000	1,000	1,000
Medium		800	400	200	100
Endangered		700	200	80	36
Critical		300	75	40	15

Results

The initialisation period *S1* in the first 15 generations lead to an average kinship of 3.4 % and on average 14.5 deleterious variants per individual, and quickly fixed 60 % of the deleterious loci. The decreased population size in *S2* led to an average kinship in *L*, *M*, *E*, and *C*, of 11 %, 39 %, 73 % and 93 %, respectively. Nearly all deleterious variants (>95%) got purged from *E*, *C* and *M*, while *L* still contained ~12 % of the initial deleterious mutations (**Figure 1**). During *S2*, in 33 % of the simulations *C*, and in 5 % *E* got extinct.

S3 clearly showed decreased proportions of viable offspring in the first generations after genetic rescue, and decreased inbreeding, when males were taken from other stocks or the cryo-reserve (**Figure 2**). Consistently across repetitions, males from the largest population or the cryo-reserve had a stronger negative impact than from smaller stocks. In 11 simulations,
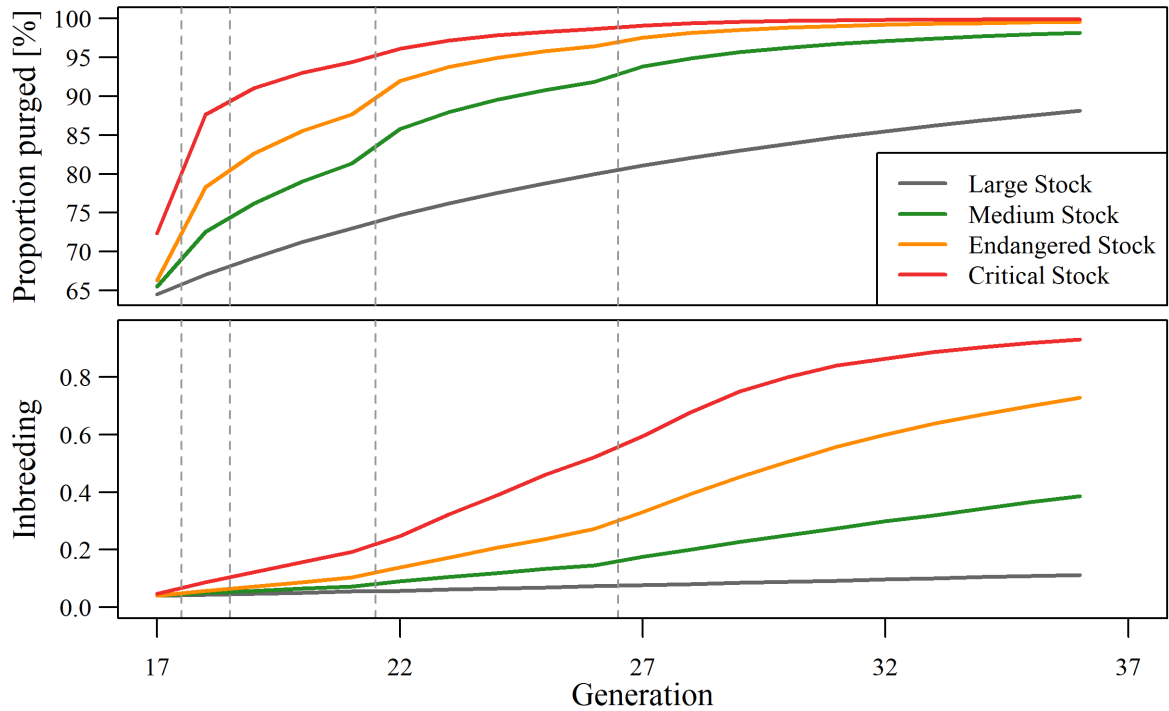
Figure 1. Top: Proportion of purged deleterious loci within a stock in *S2*, calculated on the basis of the founder generation. Bottom: Development of Inbreeding, calculated as average IBD per genotype.
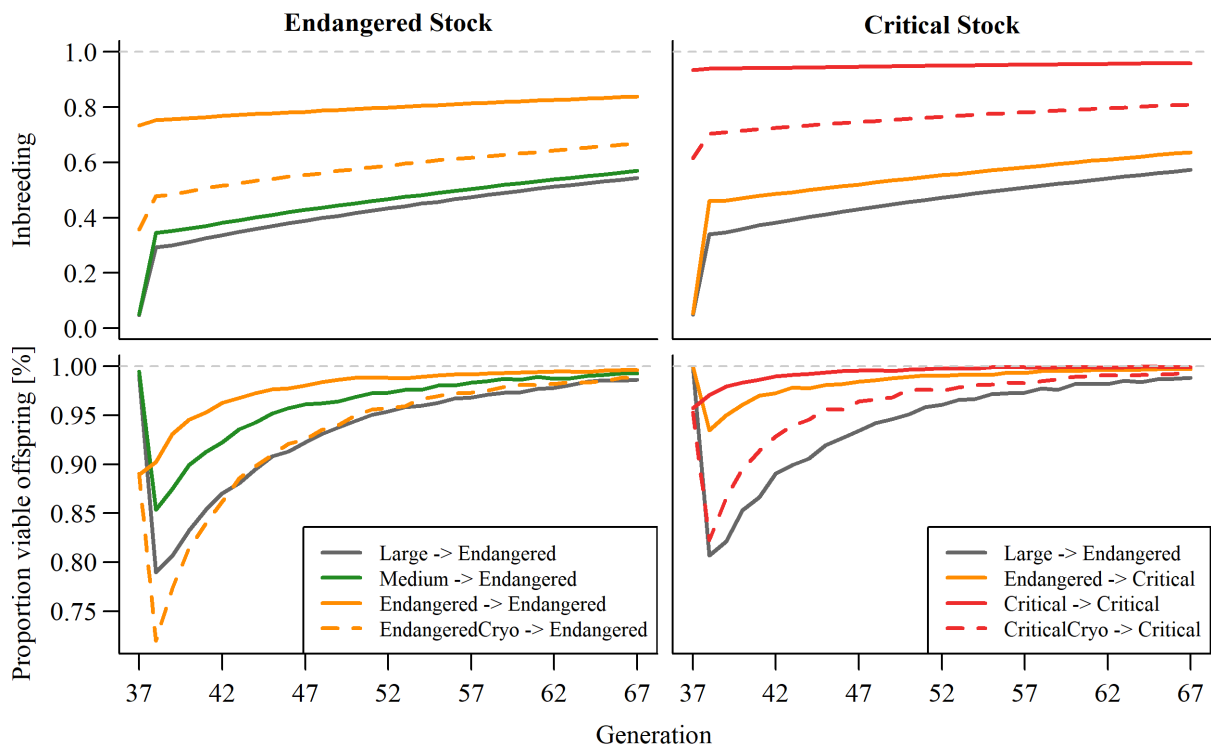
Figure 2. Degree of inbreeding derived from IBD of simulated genotypes and proportion of viable offspring for the genetic rescue scenarios of *S3* for the Endangered (N = 36) and Critical stock (N = 15).

breeding males from *E* in *C* led to extinction of *C*, likewise in each internal pairing in *C* and *E* once each. Inbreeding increase flattened and the benefit was greatest when males were taken from independent stocks rather than the cryo-reserve.

Discussion

Our results give an insight into how inbreeding and strongly deleterious variations interact in a framework of inbred livestock populations when genetic rescue is performed to restore genetic diversity. Strong inbreeding with resulting selection against deleterious variants reduced their number and frequency substantially, but genetic rescue led to decreased offspring viability due to homozygous lethal genotypes in the remaining loci in the short term. Extinction after rescue happened in *E* and *C*, but remained a rare event. Compared to the wild population scenario simulated by Kyriazis et al.(2021), genetic load in all donor stocks was limited due to fairly efficient purging. The main finding is the effect that introgression from larger sub-populations was worse compared to usage of similarly endangered stocks, as the first had a substantially higher genetic load. The detrimental effect of using gene-bank semen could be attributed to the larger historical population size at sampling and shows that careful characterisation of deleterious variation is important when using older samples. Simulation seems to be an appropriate tool to assess such effects and has been used before (Wang et al., 1999), but knowledge about the genetic structure of deleterious mutations is paramount to obtain reliable results. We studied strongly lethal variants, which might be purged as demonstrated, but variants with mild effects could become inherent (Charlesworth & Charlesworth, 1987; Hedrick & Kalinowski, 2000) and be more important in long term viability. To better account for the effect of deleterious variation in the context of conservation breeding, novel genomic approaches to assess deleterious variation, such as homozygous haplotype deficiency or sequence guided identification (Derks et al., 2021) might be of high impact when routinely employed.

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