Brief Communication

Small $N_e$ of the Isolated and Unmanaged Horse Population on Sable Island

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Abstract

For small, isolated populations 2 common conservation concerns relate to genetic threats: inbreeding and negative consequences associated with loss of genetic diversity due to drift. Mitigating these threats often involves conservation actions that can be controversial, such as translocations or captive breeding programs. Although such actions have been successful in some situations, in others they have had undesirable outcomes. Here, we estimated the effective population size ($N_e$) of the Sable Island horses to assess the risk to this population of these genetic threats. We found surprising consistency of $N_e$ estimates across the 5 different methods used, with a mean of 48 effective individuals. This estimate falls below the 50 criterion of the “50/500 rule,” below which inbreeding depression is a concern for population viability. However, simulations and knowledge of population history indicate that this population is still in its early stages of approaching equilibrium between mutation, drift, and genetic diversity; and no negative consequences have been identified that could be associated with inbreeding depression. Therefore, we do not recommend taking management action (such as translocations) at this stage. Rather, we propose continued monitoring of genetic diversity and fitness over time so that trends and any substantial changes can be detected. This represents one of the few unmanaged horse populations in the world, and therefore these data will not only alert us to serious concerns regarding their conservation status, but will also provide a wealth of information about how natural processes drive patterns of reproduction, mortality, and population growth over time.

Subject areas: Conservation genetics and biodiversity

Key words: effective population size, Equus, feral, horse, population viability, Sable Island, wildlife management

Genetic diversity is what enables a population to adapt to changing environmental conditions by providing the raw material on which natural selection can act (Frankham et al. 2002; Hedrick 2011). Therefore, populations with greater genetic diversity are better able to adapt to adverse conditions and take advantage of new opportunities in the environment (Frankham et al. 1999; Hanski and Saccheri 2006). This evolutionary potential can aid the long-term viability of a population.

Small populations will lose genetic diversity faster than large populations due to genetic drift. In addition to population size, several other factors influence the magnitude of genetic drift, including degree of isolation (Lacy 1987), mating system and degree of...
reproductive skew (Anthony and Blumstein 2000), and historic bottlenecks (Nei et al. 1975; Leberg 1992).

It is often difficult to obtain adequate data on the relative contribution of each of these factors to the risk of genetic decline in a wildlife population. The effective population size ($N_e$) is a theoretical metric developed to capture the impacts of all of these forces on patterns of genetic variation over time (Wright 1969; Leberg 2005). Thus, estimating $N_e$ is a useful means to assess the impacts of complex, and difficult to study, factors into one metric that can be estimated somewhat easily. Moreover, the size of $N_e$ directly determines how quickly genetic diversity will be lost from a population (Leberg 2005). Once an estimate of $N_e$ is available, it is possible to make inferences on the potential rate of this genetic loss and therefore identify if, and to what degree, conservation efforts are needed to mitigate this loss.

Sable Island is a small (~3400 ha), crescent-shaped island located approximately 250 km southeast of Nova Scotia, Canada. A population of horses has existed on the island since the mid-1700s. This population is not native, but rather is the result of a combination of introductions, and mixing of different breeds from the late 1700s through the 1940s (Welsh 1975; Christie 1995). The horses gained legal protection in 1961, which made it illegal to interfere with the population in any way. Thus, the horses have existed as an isolated, unmanaged population for more than 50 years, and are widely regarded as naturalized wildlife that have become an integral component of the Sable Island ecosystem. Indeed, in 2010 when it was announced that Sable Island would be designated a National Park Reserve, Parks Canada (the government agency charged with managing the park) noted the strong public opinion that the horses “are a unique and iconic feature of the island and as such their protection is of paramount importance” (Parks Canada 2010).

The population has fluctuated between ~150 and 500 individuals (Welsh 1975; Lock 1987; van Beest et al. 2014), and it is currently at an all-time high of ~534 individuals (McLoughlin P, personal communication). During the 1970s and 1980s, crashes appeared to occur in years in which particularly heavy snowfall coincided with a population size of 300 or more horses (Lock 1987).

Behaviorally, the mating system of the horses appears to resemble mate-defense polygyny, with dominant stallions controlling access to females and their young. Thus, at any given time only a small proportion of males are associated with females and are controlling access to mates.

Combined, the isolated nature of this population, its relatively small size punctuated with periodic crashes, and an apparent mating system that could dramatically reduce the functional gene pool, have raised concerns about the potential loss of genetic diversity, and therefore long-term viability, of the Sable Island horse population. To address this issue, we used genetic data to estimate $N_e$, conduct simulations to predict how genetic diversity will change over time, and interpret these results in the context of the long-term conservation of this population.

Materials and Methods

Since 1987, tissue samples have been collected from carcasses of horses that have died of natural causes (Clare et al. 2015). Based on observational monitoring by one of us (ZL), carcasses have been found for the majority of horses that have died during this time. We were interested in how genetic diversity has changed over time, which can then be used to estimate $N_e$. Therefore, we analyzed all samples from the earliest 2 years for which samples were available (1987–1988, $n = 55$), and samples found in 2011 ($n = 77$), the latest year for which samples were available at the time of this study, for a total of 132 samples. At an average generation time of 4 years (Plante et al. 2007), this 24-year time span represents approximately 6 generations.

Genetic Analyses

DNA was extracted using phenol/chloroform procedures (Sambrook and Russell 2001; Clare et al. 2015). Samples were amplified at 17 microsatellite loci using the StockMark®s Equine Genotyping Kit (Applied Biosystems, Foster City, CA). An 11 μL reaction volume was used containing the following: 1.61 μL StockMark®s PCR buffer, 2.57 μL dNTP mix, 0.32 μL AmpliTaq Gold® DNA polymerase, 2.57 μL amplification primer mix, 1.93 μL reaction water, and 10ng DNA. For each reaction, positive and negative controls were also amplified. The positive controls consisted of 2 μl of a 1:80 dilution of the control DNA provided with the kit, and 2 μL of TF$_{rec}$ was used for negative controls. PCR cycling conditions consisted of: 95 °C for 10 min; 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min; and a final extension step of 72 °C for 1 h. PCR products were prepared for capillary electrophoresis by adding 2–10 μL of HiDi™ formamide and 0.25 μL GeneScan™ 600 LIZ® size standard (Applied Biosystems). Amplified products were then size-separated and visualized on a 3500xl Genetic Analyzer (Applied Biosystems). Two positive control samples were included on each plate to ensure consistent genotyping across plates and time, as well as to assess genotyping error rates.

Samples were genotyped using the GeneMarker® software (SoftGenetics, State College, PA). Estimates of locus characteristics and variability were obtained using cverus ver. 3.0.6 (Kalinowski et al. 2007), and genotypes were tested for deviations from Hardy–Weinberg equilibrium using GENEPOP v.2.2.2 (Rousset 2008). Loci were not tested for linkage, because this kit was generated based on loci that were known to not be linked.

Estimating $N_e$

There are multiple methods available to estimate $N_e$ from genotype data, which can be divided into 2 primary approaches: 1) temporal methods that require samples from 2 different time points; and 2) single sample methods that estimate $N_e$ based on genetic characteristics from samples at only one point in time. Although our primary goal was to obtain an $N_e$ estimate for the Sable Island horse population, we also wanted to test the consistency of estimates across different methods. Therefore, we compared estimates of $N_e$ based on 5 different methods. Additionally, for the single-sample approaches, estimates were obtained for each time period independently.

Two temporal methods were used to estimate $N_e$: one based on changes in heterozygosity over time; and the other based on fluctuations in allele frequencies over time. In an ideal Wright-Fisher population there is a direct relationship between $N_e$ and the rate at which heterozygosity declines (Wright 1931; Hedrick 2011). An estimate of $N_e$ based on this method was obtained by manually comparing observed heterozygosity ($H_o$) between samples from the 2 time periods. The second temporal method is based on the fact that the degree to which allele frequencies fluctuate from one generation to the next is directly related to $N_e$ (Waples 1989). Estimating $N_e$ from these fluctuations requires the calculation of the standardized variance in allele frequencies ($F_o$), for which we used the $F_o$ calculation of Jorde and Ryman (2007). For these analyses a minimum allele frequency
of 0.02 was used, and confidence intervals were obtained using the “parametric” method in NeEstimator v2 (Do et al. 2014), where the number of independent alleles is used as the degree of freedom in a Chi-square distribution.

Three different single sample methods were used to estimate \( N_e \): a sibship reconstruction method, an approximate Bayesian technique, and the linkage disequilibrium (LD) method. The sibship reconstruction method was implemented using the program COLONY (Wang 2004; Wang and Santure 2009), and is based on the idea that as \( N_e \) declines the number of siblings in a random sample from a population will increase (Wang 2009). This method was implemented assuming allelic dropout and genotyping error frequencies of 0.01 and 0.05, respectively. Sibship reconstruction was based on the pairwise likelihood score method, which is faster than the full likelihood method while still maintaining high accuracy.

With the approximate Bayesian approach implemented in ONEsAMP (Tallmon et al. 2008), 8 summary statistics are calculated for the genetic data set. Then, 50,000 simulated populations are generated based on parameters specified by the user to resemble the sampled population. To generate each simulation, an \( N_e \) is randomly drawn from a user-specified range. An estimate of \( N_e \) is then based on analyses of how closely the summary statistics from the simulated populations with different \( N_e \) values resemble those from the actual data set (Beaumont et al. 2002; Tallmon et al. 2004a, 2008). These analyses were run for each time period, using lower and upper ranges for \( N_e \) of 10 and 500, respectively.

Lastly, estimates of \( N_e \) based on the LD method were obtained for each time period (Hill 1981; Waples 1990, 2006; Peel et al. 2013) using NeEstimator (Do et al. 2014). This approach is based on the idea that as \( N_e \) declines, LD will decrease (and the appearance of linkage will increase) due to increasing departures from expected genotype and gametic frequencies (Hill 1981). A minimum allele frequency of 0.02 was used, and confidence intervals for this estimate were obtained using the parametric method in NeEstimator, where the number of independent alleles is used as the degree of freedom in a chi-square distribution.

Simulations of Expected Decline in Genetic Diversity
We used the program EASYPOP to conduct simulations based on our estimates of \( N_e \) to predict how genetic diversity will change over time (Balloux 2001). Specifically, we simulated one randomly mating population containing 24 females and 24 males (see Results section) genotyped at 10 loci with a mutation rate of \( 5 \times 10^{-4} \) mutations/generation where 92% of mutations followed the single stepwise mutation model (SSM) and 8% could result in any of 100 possible allele states (the K-Allele model). This mutation scheme is generally consistent with the data currently available on the characteristics of microsatellite mutations (e.g., Weber and Wong, 1993; Di Rienzo et al. 1994). Populations were started with the maximal variation and the simulations were run for 5000 generations to allow the populations to reach mutation-drift equilibrium. Twenty-five iterations were conducted to assess the variability between runs. It should be noted that these simulations provide a conservative (i.e., “best case scenario”) estimate of the rate at which genetic diversity will be lost because they assume random mating, whereas the true mating system is likely quite polygynous. However, the degree of polygyny, and relative variance in male versus female reproductive success, are not currently known, and therefore could not be accounted for appropriately in the simulations.

Data Archiving
In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying these analyses (genotypes of individuals) on Dryad.

Results
Microsatellite Genotyping
Of the 17 loci that were amplified with the StockMarks Equine Genotyping Kit, 4 were discarded due to ambiguity in the interpretation of the electropherograms (loci HMS1, HTG6, HTG7, and HTG10). Genotypes at another 3 loci significantly deviated from Hardy–Weinberg expectations (loci ASB23, HMS3, and LEX3), and were therefore removed from subsequent analyses. Characteristics of the remaining 10 loci are shown in Table 1.

Estimates of \( N_e \)
The average observed heterozygosity of the population in 1987–1988 was 0.583, and that in 2011 was 0.548. This decline in heterozygosity over a time period of 6 generations results in an \( N_e \) estimate of 49.7, using the temporal method based on changes in heterozygosity over time. The temporal method based on fluctuations in allele frequencies over time resulted in an \( N_e \) estimate of 49.4 (95% CI 27.3–70.4 using 0.02 as the lowest allele frequency considered).

For the single sample methods of estimating \( N_e \), we obtained estimates for both time periods independently. The coancestry analysis of COLONY resulted in \( N_e \) estimates of 25 (95% CI 15–44) and 25 (15–43), for 1987/1988 and 2011, respectively. The estimates from ONEsAMP were 48.6 (37.4–89.5) and 30.9 (23.4–58.3). Lastly, the estimates using the LD method were 86.4 (42.7–433.3) and 64.6 (40.9–120.3).

These estimates are all fairly consistent with one another, and result in an overall mean \( N_e \) estimate of 47.5 individuals (combining the temporal and single sample estimates) (Figure 1).

Simulations of Expected Decline in Genetic Diversity
The simulations indicate that a population with an \( N_e \) of 48 effective individuals will lose genetic diversity rapidly, reaching a mutation-drift heterozygosity equilibrium of ~0.065 after about 500

Table 1. Characteristics of the 10 microsatellite loci used for estimating \( N_e \)

| Locus | \( A \) | \( H_o \) | \( H_e \) |
|-------|-------|-------|-------|
| AHT4  | 5     | 0.722 | 0.677 |
| AHT3  | 4     | 0.521 | 0.614 |
| ASB2  | 4     | 0.381 | 0.409 |
| ASB17 | 9     | 0.811 | 0.834 |
| CA425 | 3     | 0.646 | 0.539 |
| HMS2  | 2     | 0.139 | 0.144 |
| HMS6  | 7     | 0.735 | 0.763 |
| HMS7  | 3     | 0.441 | 0.563 |
| HTG4  | 3     | 0.648 | 0.656 |
| VHL20 | 6     | 0.581 | 0.612 |
| Mean  | 4.6   | 0.563 | 0.581 |

Included is the locus name, number of alleles (\( A \)), observed heterozygosity (\( H_o \)) and expected heterozygosity (\( H_e \)).
generations (Figure 2). The population currently has an average heterozygosity of 0.548, suggesting that it has only recently been reduced to a small \( N_e \), and is early in the stages of genetic decline. Indeed, this level of heterozygosity is expected after \( \sim 72 \) generations, or 288 years based on a 4-year generation time (Figure 2). Although the starting level of heterozygosity is not known, it was certainly less than 1, as was required for the simulations. However, the estimated time frame is consistent with what is known about the introduction of horses to the island, with an initial introduction approximately 250 years ago (between 1738 and 1760), with subsequent “enhancement” by other stocks periodically through 1961 (Christie 1995).

Discussion

The analyses show that the \( N_e \) of the Sable Island horse population is small, with an average estimate of \( \sim 48 \) effective individuals. Although there were differences among estimates obtained using different methods, these were smaller than expected based on other studies. For example, in a comparison of estimator performance on matterjack toads (\textit{Bufo calamita}), Beebee (2009) found striking differences between estimates obtained using different methods, with some differing by an order of magnitude or more. Thus, the relatively small variation across methods suggests that our estimate is robust.

This estimate for \( N_e \) falls below the 50 criterion for short-term \( N_e \) of the “50/500 rule” in conservation genetics. Briefly, Franklin (1980) proposed that for populations to remain sustainable, the short-term \( N_e \) should be greater than 50 individuals and the long-term \( N_e \) should be greater than 500 individuals. These numbers are based on general principles for reducing the likelihood of loss of genetic variation and subsequent negative consequences and increased risk of extinction due to inbreeding depression and genetic drift, respectively (Franklin 1980; Jamieson and Allendorf 2012; Frankham et al. 2014). While never proposed as a strict set of criteria below which conservation actions are futile, the 50/500 rule is still useful as a signpost for identifying when \( N_e \) numbers may be falling to concerning levels, and thus when conservation actions to minimize the loss of genetic diversity may be appropriate.

Although average heterozygosity is currently fairly high (0.548), the simulations suggest that the population will lose heterozygosity rapidly (by \( -1/2N_e \) or 0.01 per generation). The obvious question, then, is what should be done if maintaining the horse population is a management goal for the Sable Island National Park Reserve? Two options are to: 1) develop a plan for the “genetic enhancement” of this population by introducing horses from other locations; or 2) take no management actions, but continue to monitor the population to assess genetic diversity, inbreeding, and fitness over time, and postpone any decision making (or enactment of a predeveloped plan) until a genetic reduction in fitness is suspected. Both options have merit as well as pitfalls.

There are several examples where translocations have successfully introduced new genetic diversity into a population, and subsequently increased genetic diversity and reduced or eliminated the negative consequences of inbreeding (e.g., Westemeier et al. 1998; Pimm et al. 2006). Numerous horses have been translocated to Sable Island in the past, and given that transportation and veterinary care of horses are routine and well-developed, the chance of success would likely be high. However, the potential introduction of new pathogens and the dilution of local adaptations (e.g., Tallmon et al. 2004b; Hedrick and Fredrickson 2010).

When transporting animals from one location to another, there is always a chance that the relocated individuals are carrying pathogens not present in the new location. Since the immune systems of animals in this new location have not adapted to these pathogens, the results can be devastating (e.g., Cunningham 1996; Carbyn and Watson 2001). The horses on Sable Island have been completely isolated from mainland populations for over 50 years, with only periodic contact with a relatively small number of horses from the mainland for the preceding 250 years. Thus, it is possible that the pathogens present in the Sable Island horses are not representative of those circulating in mainland populations, and that the translocation of new individuals would also unintentionally translocate new pathogens.

Additionally, the horses on Sable Island have undoubtedly adapted to their localized conditions, such as living in an environment in which they lack protection and are exposed to high winds, precipitation, and blowing sand; and, perhaps most importantly, having to sustain themselves on plants characteristic of coastal dune systems that go through seasonal changes in abundance/availability.
These conditions clearly represent strong selection pressures as represented by the periodic population crashes, primarily after harsh winters. The cumulative effects of this selection has shaped the alleles and adaptations of this population, and the translocation of mainland horses (and their alleles) could dilute and disrupt these adaptations and therefore have the unintended consequences of reducing fitness and increasing extinction probabilities (e.g., Greig 1979; Marshall and Spaldon 2000; Tallmon et al. 2004b).

Given these concerns, we do not recommend translocations to remedy this low Nₑ at this stage. Instead, we propose continued monitoring of genetic diversity and fitness (reproductive and mortality rates) over time so that trends can be monitored and any substantial changes can be detected. Although translocations may be appropriate in the future, we also recognize that genetic decline is not necessarily inevitable: there is increasing evidence that, at least in some small populations, mate choice decisions and/or biased fertilization patterns can reduce the effects of genetic drift and maintain genetic diversity over time (e.g., Bensch et al. 2006; Frasier et al. 2013). It is not yet known if such a mechanism is acting in this population, but such analyses represent one of our future research goals. Continued monitoring of the population will not only identify if and/or when translocations may be necessary, but in the meantime will also provide a wealth of data on the biology of small populations. Being one of the few isolated and unmanaged horse populations in the world, the Sable Island horses represent a valuable opportunity to learn about the processes that drive patterns of reproduction, mortality, and population growth over time.

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