

Revealing life-history traits by contrasting genetic estimations with predictions of effective population size

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Abstract

Effective population size, a central concept in conservation biology, is now routinely estimated from genetic surveys, and can also be theoretically-predicted from demographic, life-history and mating-system hypotheses. However, by evaluating the consistency of theoretical predictions with empirically-estimated effective size, insights can be gained regarding life-history characteristics, as well as the relative impact of different life-history traits on genetic drift. These insights can be used to design and inform management strategies aimed at increasing effective population size. Here we

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describe and demonstrate this approach by addressing the conservation of a reintroduced population of Asiatic wild ass (*Equus hemionus*). We estimate the variance effective size (N_{ev}) from genetic data ($N_{ev} = 24.3$), and we formulate predictions for the impacts on N_{ev} of demography, polygyny, female variance in life-time reproductive success, and heritability of female reproductive success. By contrasting the genetic estimation with theoretical predictions, we find that polygyny is the strongest factor effecting genetic drift, as only when accounting for polygyny were predictions consistent with the genetically-measured N_{ev} , with 10.6% mating males per generation when heritability of female RS was unaccounted for (polygyny responsible for 81% decrease in N_{ev}), and 19.5% when it was accounted for (polygyny responsible for 67% decrease in N_{ev}). Heritability of female reproductive success was also found to affect N_{ev} , with $h_f^2 = 0.91$ (heritability responsible for 41% decrease in N_{ev}). The low effective population size is of concern, and we suggest specific management actions focusing on factors identified as strongly affecting N_{ev} —increasing the availability of artificial water sources to increase number of dominant males contributing to the gene pool. This approach – evaluating life-history hypotheses, in light of their impact on effective population size, and contrasting predictions with genetic measurements – is a general, applicable strategy that can be used to inform conservation practice.

Introduction

Effective population size is a fundamental concept in conservation biology, as it allows quantifying genetic drift in non-ideal populations in a comparable manner. Such measures are crucial for monitoring and for risk-assessment of small and endangered populations (Schwartz et al. 1998; Luikart et al. 2010; Allendorf et al. 2012). An effective size of a population denotes the size of an ideal population that experiences the same levels of genetic drift with respect to a certain population-genetic measure (Templeton 2006). The concept of effective population size has been applied in conservation and management in two different ways: 1) estimation of effective sizes from

genetic data, and 2) prediction of effective sizes from demographic and life-history data and hypotheses.

Estimation of effective sizes from genetic data can be done using a single sample of the population, most commonly by analyzing linkage disequilibrium, or, alternatively, by temporal sampling at two sufficiently distant points in time (Schwartz et al. 1998; Luikart et al. 2010; Wang et al. 2016).

Temporal samples from populations of conservation-concern are more difficult to procure, since they require genetic monitoring over several generations; however, these methods are considered more precise and more robust to violation of assumptions than estimates derived from a single generation (Leberg 2005; Wang et al. 2016). Predictions based on demographic and life-history data are most often made by evaluating analytic formulations of the deviation from the assumptions of an ideal population, providing predictions of expected effective population sizes for different aspects of genetic drift. For example, fluctuating population size, uneven sex-ratios, un-even distribution of reproductive success (henceforth RS), are all violations of the assumptions of idealized populations, and all can be accounted for in their impact on effective population sizes (Templeton 2006; Wang et al. 2016).

These two approaches for evaluating effective population sizes — genetic estimation and theoretical prediction — are most often employed separately, either to monitor the level of genetic drift using genetic samples, or to provide conservation-relevant predictions based on life-history traits.

However, by adopting a strategy combining the two, it is possible to gain additional (and important) insights on wild populations — the direct genetic estimation could provide the “ground-truth” with which hypotheses regarding life-history, ecology and behavior can be evaluated. This strategy can provide an informative account of how different life-history traits impact effective population size and genetic drift, and improve our comprehension of which factors actually matter, in a conservation context, and to what extent. Despite its advantages, a detailed study describing such a combined approach and highlighting its benefits for conservation has not been fully presented.

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Here we methodically describe how contrasting genetic estimates with theoretical predictions based on demography and life-history traits can reveal life-history characteristics of populations and their relative impacts on genetic drift. Moreover, we show how these insights can be used to inform management aimed at increasing effective sizes. We demonstrate the applicability of this approach by addressing the management of a population of conservation concern — the Asiatic wild ass (*Equus hemionus*) in the Negev Desert, Israel. The Asiatic wild ass was once abundant throughout Western and Central Asia, but has largely been extirpated from its native range, including from the Negev desert (Kaczensky et al. 2015). The species was successfully reintroduced to the Negev, and genetic samples were collected at two time-points, three decades apart, allowing for a relatively accurate estimation of the variance effective population size, N_{ev} , by analyzing the accumulation of changes in allele frequencies due to genetic drift (Nei & Tajima 1981; Wang 2005; Wang et al. 2016).

Previous studies have shown that the wild ass Negev population exhibits fission-fusion social structure (Rubenstein 1994; Saltz et al. 2000). In fission-fusion social structure, unlike in harem-polygyny observed in several other equids, females are not constrained to mate with the same dominant male in consecutive breeding seasons. The females form non-stable groups that split and join on occasion, bachelor males form small groups, and the dominant males remain solitary and defend territories. Using genetic and behavioral data, it was concluded that the population is highly polygynous, with less than 25% of the males participating in the mating process each generation (Renan et al. 2015). Polygyny at this level is expected to increase genetic drift and decrease effective sizes, but other life-history traits may play an important role as well, particularly the variance in RS of females (Nunney 1996). Moreover, although rarely demonstrated in wild populations, heritability of RS, either genetically or through social inheritance, may also significantly affect genetic drift, and therefore its impact on genetic drift should be considered (Robertson 1961; Nei & Murata 1966).

In order to demonstrate the application of our suggested approach, and better understand genetic drift in the wild ass population, we compared genetically-estimated N_{ev} with several different

theoretical predictions of N_{ev} , each corresponding to a plausible hypothesis regarding life-history and mating-system characteristics. The genetic data was obtained from blood, tissue and fecal samples from two points in time for the N_{ev} estimation, and parameterized life-history predictions using observations from a long-term monitoring project. Contrasting effective-size predictions with empirically-estimated effective-size allows us to gain insights both on the life-history of the population and on its impact on genetic drift, with implications for conservation and management. This strategy is applicable in a wide range of conservation scenarios where genetic information is available, as the theoretical predictions and hypotheses can be tailored to the issues relevant to each case.

Methods

Between 1982-1987, 28 wild asses were introduced from a captive population into the Negev, and 10 more were released in 1992-1993. The population was intensively monitored during reintroduction (Saltz & Rubenstein 1995; Saltz et al. 2000, 2006). In 2012, four generations after its reintroduction (Asiatic wild ass generation length is approximately 7.4 years; (Saltz & Rubenstein 1995)), a non-invasive genetic survey was conducted to evaluate the genetic composition of the current population (Renan et al. 2015).

In order to understand how different life-history characteristics affect genetic drift in this population, we compared and contrasted different hypotheses regarding demography, life-history, and mating-system with genetic measurements. First, we formulate predictions of N_{ev} based on the known life-history and social structure of the population. Specifically, we consider the effects of demography, polygyny, and female variance and heritability of RS (parameter/notation summary in Table 1). We base the formulations and their parameterization on previous studies and detailed monitoring records. Second, we estimate N_{ev} using genetic data. Finally, we compare the

predictions with the genetic measurements, and determine whether our predictions coincide with the genetically-measured N_{ev} .

Predictions of

Demography

The null hypothesis we consider is that life-history and mating-system effects are negligible, and only demography (including sex-ratios) affects genetic drift. For this purpose we calculate the variance effective size that incorporates only direct demographic effects (including sex-ratio skew), N_d . At reintroduction and during the first generation the population was well-monitored, all individuals were accounted for, and precise census numbers were obtained (Saltz & Rubenstein 1995). For the next three generations, census sizes were estimated by extracting the growth rate from a life-history table (Saltz & Rubenstein 1995), estimated to be $R_0 = 1.87$ per generation (Renan et al. 2015). We assumed a margin of error of $\pm 10\%$ of this R_0 , and calculated the predicted census size range for each generation. An effective population size considering non-even sex ratios, for each generation, was calculated as (Wright 1931):

$$\frac{4N_m N_f}{N_m + N_f}$$

where N_m and N_f are the number of males and females in that generation, respectively. The overall effective size, \bar{N}_d , was calculated by taking the harmonic mean of the N_d for each generation after the initial reintroduction (i.e., generations 1-4 after reintroduction; N_d incorporates only the offspring generations; (Templeton 2006)).

Polygyny

The polygynous nature of the Asiatic wild ass mating-system may have a strong effect on genetic drift in the population (Renan et al. 2015). Polygyny can be modeled in various ways, but when

males can be partitioned into two classes — 'dominant' and 'non-dominant' — as is the case in equids, a common approach is to evaluate the proportion of mating males (p_0) per generation (Wade & Shuster 2004).

This means that, effectively, only $p_0 N_m$ males participate in the mating process, and the effective population size for each generation accounting for demography and polygyny, N_p , can be stated as a reformulation eq.1:

$$N_p = \frac{4p_0 N_m N_f}{p_0 N_m + N_f} \quad (2)$$

The overall N_{ev} accounting for demography and polygyny, $\overline{N_p}$, is obtained by taking the harmonic mean of the sizes in generations 1-4, as explained above. Note that eq.2 describes the effect of polygyny in a fission-fusion social structure, where females are not constrained to mate with a single male, as is the case in this wild ass population, whereas for harem-polygyny a different formulation should be used (see Wang et al. 2016).

Female variance in RS

The female contribution to genetic drift may also be important even in polygynous mating-systems, most commonly through variance in life-time reproductive success (ltRS) (Nunney 1996). In an ideal population, females are considered to have equal fitness, and variance in RS follows a Poisson distribution (with a mean and variance of 2 in stable populations). If female ltRS is over-dispersed compared to a Poisson distribution (i.e., $variance > mean$), the few individuals with higher RS will be more genetically represented in the following generation. This increases genetic drift and decreases the effective population size, relative to the ideal case (Nunney 1996).

Under the assumption of equal sex-ratios (adjusted for in eq.1 and eq.2) and no correlation between offspring sex and RS, the effect of variance in ltRS on N_{ev} (relative to an ideal size N), denoted as N_r , can be partitioned to male (v_m) and female (v_f) variance components (Wang & Hill 2000):

$$N_r = \frac{4N}{2 + \frac{1}{2}v_m + \frac{1}{2}v_f} \quad (3)$$

Since we already considered polygyny in eq.2, v_m refers to the variance in ltRS in dominant males; we will assume that there are no major fitness differences among dominant males, i.e., $v_m = 2$.

Here it will be useful to parameterize eq.3 by empirically estimating v_f . Data regarding female ltRS are not easily attained in long-lived species, however we were able to evaluate the ltRS distribution by examining detailed records taken during 1985-1996 (Table S1). These records were attained during an intensive monitoring program which included routine observations (3 times a week) of the population (Saltz et al. 2000). All adult females in the population were individually recognized, and each birth event was noted. Newborns were continuously monitored until adulthood, and were also individually recognized.

Examining these records, we noted the number of offspring of each female and the number of reproductive years during the records period. The number of reproductive years was defined as the number of years above the age of 3, the typical age for onset of reproduction in wild ass (Saltz & Rubenstein 1995). We then estimated the annual reproductive success, aRS, for each female; we discarded all females for which we had <3 reproductive years in the records, since wild ass sire at most one offspring per year, and estimating aRS based on just one or two years may be non-indicative of the actual numbers.

In order to evaluate female ltRS, we also needed to know the number of reproductive years for females. We did not have detailed data of life expectancy (and, hence, number of reproductive years) for each individual female, since many females were still alive when the records ended.

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However, since life-history tables of the population are available (Saltz & Rubenstein 1995), we estimated the average life expectancy, conditioned on reaching an age of six (females that did not reach this age would not be included in the study since we selected only females with >3 reproductive years). This we used to derive the average number of reproductive years, and then to calculate l_{tRS} for each female, and the overall mean and variance of l_{tRS} . For consistency, we used the same life-expectancy estimate even if we knew the exact life-time of a specific female. Since this procedure does not account for variance in life-expectancy (of females aged >6), our estimate of l_{tRS} is probably an underestimate, and we use it only to broadly characterize the l_{tRS} distribution and estimate v_f .

Heritability of RS

Another life-history trait that may induce genetic drift, less documented in wild populations and less considered in the context of conservation, is the “heritability” of RS (Robertson 1961; Nei & Murata 1966). In our case RS is said to be “heritable” (at least partially) when there is a significant, positive regression between RS of mothers and the RS of their daughters. Such a positive regression could arise either genetically (the usual meaning of heritability) or through non-genetic mechanisms such as social inheritance. Regardless of the cause of RS heritability, alleles found in individuals with high RS, even those not associated with RS-related phenotypes, will experience increased representation in the population until their association with high RS dissipates. This constant genome-wide pressure will increase the over-all rate at which gene-lineages are lost or fixed in the population, even in neutral loci, i.e., increased genetic drift and reduced effective sizes will be observed. While for those loci associated with the inherited RS this process may be beneficial in the context of conservation (perhaps leading to adaptation), the overall genetic diversity is expected to decrease when such heritability is high.

In order to predict the effect of heritability on N_{ev} , we used the formulation of Nomura (2002), who expanded on the formulations of Nei and Tajima (1981). This formulation considers separate components of heritability of RS for the two sexes, h_m^2 and h_f^2 for paternal and maternal inheritance, respectively. The assumption is that inherited RS is expressed only when the sex of the parent and the sex of the offspring are the same (e.g., females inherit RS from their mothers and not from their fathers). In this case, the effect of heritability on N_{ev} due to inherited RS (relative to an ideal size N), denoted as N_h , can be expressed as (Nomura 2002):

$$N_h = \frac{4N}{2 + v \left(1 + \frac{3}{2} (h_m^2 + h_f^2 + 2r_g h_m^2 h_f^2) \right)} \quad (4)$$

where r_g is the correlation between the contribution of male and female parents, and v is the overall variance in ItRS.

To make use of eq.4, we first must estimate the heritabilities by parent-offspring regression. For this purpose, however, life-history data spanning at least three generations are needed, very rarely available in wild mammals. The 1985-1996 records of the Negev wild ass population were, however, sufficient to allow for a mother-daughter regression analysis. We noted all mother-daughter pairs for which we had at least three years of records for both, and performed regression of mother's aRS vs. the mean aRS of her daughters to estimate h_f^2 . We had no observational data regarding h_m^2 , hence we assumed $h_m^2 = 0$, keeping in mind that we are only quantifying maternal heritability of RS, and if paternal heritability is significant it would constitute an additional factor reducing N_{ev} (see *Discussion*).

Estimation of N_{ev} from temporal genetic data

The genetic data we analyzed are composed of samples taken at two points in time — during reintroduction and in 2012. Allele frequencies were estimated for 8 microsatellite markers from

blood samples that have been kept frozen since 1991 for the founding population, and from the non-invasive genetic survey for the 2012 population. The genetic survey was conducted throughout the geographic range of the population distribution. Allele frequencies and sample sizes can be found in Table 2, and details regarding sample collection, DNA extraction and the genotyping procedure can be found in Renan et al. (2015).

Temporal genetic sampling allows for the evaluation of N_{ev} by considering accumulation of changes in allele frequencies due to genetic drift in the four generations that separate the samples. We estimated N_{ev} using two methods: F-statistics following Nei and Tajima (1981), and a Maximum pseudo-likelihood method using the software MLNE (Wang 2001). Since the results are qualitatively similar, and there is evidence that maximum likelihood methods are more precise (Wang et al. 2016), we present the F-statistics analysis in *Appendix S1*.

Although there is evidence for the formation of population structure in the population (Gueta et al. 2014), this estimation of N_{ev} should be robust to the existence of population structure if sampling is not biased to specific subpopulations (Wang et al. 2016), as is the case with our sampling scheme.

The existence of overlapping generations may also introduce bias to N_{ev} estimations. Accounting for effects of overlapping-generations on genetic drift can be similarly to other life-history characteristics described above. Nevertheless, overlapping-generations models are typically complex, and require detailed accounts of demographic parameters of the different life-stages, which are not often available for wild populations. However, with four generations between samples, and for species with type I survivorship (low fecundity rates and parental care) such as Asiatic wild ass, overlapping-generations biases should be minimal (Waples & Yokota 2007)

Contrasting N_{ev} estimation with predictions

We have two approaches for evaluating N_{ev} : one based on life-history data, and one based on genetic data. Since the primary definition of an effective size is based on its genetic impact, we

consider the genetic estimator as the “ground-truth”, and evaluate whether the predictions from life-history data match the genetic estimation. We therefore evaluate the life-history hypotheses underlying N_{ev} predictions, thus better understanding the life-history of the wild ass population, as well as the effect of life-history traits on genetic drift.

Described above are predictions for the effect of genetic drift given several relevant life-history traits: demography ($\overline{N_d}$), parameterized by observations; polygyny ($\overline{N_p}$), with parameter p_0 ; female variance in lRS (N_r), parameterized by observation data; and heritability of female RS (N_h), parameterized by mother-daughter regression analysis (Table 1). Each of these traits alone, as well as their different combinations, provide predictions for N_{ev} . We treat each combination of traits as a hypothesis, and by comparing N_{ev} predictions under different hypotheses with the estimated N_{ev} we can determine which combination of traits (and their impact on genetic drift) is consistent with observed genetic drift, and which hypotheses are not. All hypotheses we considered included the effect of demography on genetic drift, since census sizes are the basis from which effective size predictions are formulated.

Results

Predictions of N_{ev}

The census numbers and demographic-predicted N_d for each generation are presented in Table 3. The overall demographic-predicted N_{ev} , for the time period between reintroduction and the time of the non-invasive genetic survey, is $\overline{N_d} = 120.9$ (with error margins 114.7-126.5).

The effect of polygyny, $\overline{N_p}$ is determined by the parameter p_0 . Following the findings of Renan et al. (2015) that <25% males participate in the mating process, we considered the range $0 \leq p_0 \leq 0.25$ and quantified the effect of polygyny for all these p_0 values (Fig.1). Considering the effect of

polygyny alone (and demography) on genetic drift, effective size is predicted to be $\overline{N_p} \leq 48.3$ (with error margins 45.8-50.6 on this bound).

We had reproductive success data for 35 females (Table 4). The average life-expectancy of females aged > 6 calculated from life-history table is 12.6, and therefore we considered an average of 10.6 reproductive years for the calculations of ItRS. The mean ItRS of the 23 females with sufficient data is 6.11 (SE ∓ 0.51), and the variance is 6.05 (SE ∓ 0.33). The variance-to-mean ratio is 0.99, very close to the expected value of 1 under a Poisson distribution (variance-to-mean ratio range given estimated errors is 0.88-1.16). Therefore, female ItRS is approximately Poisson-distributed, and the female variance in ItRS, after accounting for population growth, is $v_f \approx 2$. This implies that female variance in ItRS is not predicted to significantly induce or reduce genetic drift ($N_r \approx N$; eq.3).

Ten mothers had daughters with information regarding RS (Table 4). The mother/mean-daughter RS regression analysis (Fig.2) indicated significant heritability ($p < 0.05$) with $h_f^2 = 0.91$ ($n = 10$; $y = 0.91x - 0.12$; $R^2 = 0.54$; $p = 0.016$; $SE \mp 0.30$). Since this is a mother-offspring regression, and not a classic parent-offspring regression, $h_f^2 > 0.5$ implies that the mode of inheritance is maternal, since otherwise the overall heritability factor will be larger than one and nonsensical. Therefore, the assumption of Nomura (2002) that females inherit RS from their mothers alone is a valid assumption, and eq.4 applies. Taking the total ItRS in the mating population as $v = 2$ (since $v_f \approx 2$ and we assume $h_m^2 = 0$), we quantify the effect of heritability of female ItRS on genetic drift (eq.4) as $N_h = 0.59N$ ($0.57N-0.69N$, consideration estimation error and $h_f^2 \leq 1$), i.e. heritability of female ItRS reduces N_{ev} by a factor of 0.59.

Estimation of N_{ev} from genetic data

The allele frequencies at the two time-points are presented in Table 2. The Maximum pseudo-likelihood point estimate was $N_{ev} = 24.3$ (95%CI 13.8-44.0). F-statistics estimates were between $N_{ev} = 17.4$ and $N_{ev} = 21.9$, depending on p_0 (details in *Appendix S1*).

Contrasting N_{ev} estimation with predictions

Comparing N_{ev} predictions under different hypotheses with N_{ev} estimation allows us to determine which hypotheses are consistent with the observed genetic drift and which are not, summarized in Fig.1. Variance in female lRS was not found to substantially affect the prediction of N_{ev} ($N_r \approx N$) and therefore was not directly used to formulate any life-history hypothesis. The hypothesis that only demography is driving genetic drift ($\bar{N}_d = 120.9$; Fig.1, green) and the hypotheses that only demography and the measured heritability of RS ($N_h = 69.1$; Fig.1, red) are responsible, were both inconsistent with the observed genetic data (Fig. 1 and Fig. SI1, purple). Given the plausible sources of genetic drift considered here, this implies that polygyny cannot be discounted as a source for genetic drift. With only demography and polygyny, taking the Maximum-likelihood N_{ev} estimate, the point estimate for the level of polygyny is $p_0 = 0.106$, with error range of 0.10-0.11 (Fig.1, blue). With demography, polygyny and heritability of RS, the estimates are higher, with a point estimate at $p_0 = 0.195$ and a range of 0.16-0.22 given estimation errors (Fig.1, orange). This means that we estimate that 10.6% of the males participate in mating, considering the genetic and demographic data, and that this estimate would be 19.5% if we also account for the estimated level of RS heritability. For the F-statistics N_{ev} estimates, considering demography and polygyny results in an estimated range of $p_0 = 0.08$ to 0.10, and for demography, polygyny and heritability of RS the estimated range is $p_0 = 0.12$ to 0.19 (Fig.SI1).

Discussion

Comparison of N_{ev} predictions and the actual genetic estimation allows understanding of the different plausible components affecting genetic drift. In the wild ass population, the hypothesis that

demography alone, unmodulated by life-history or mating system, determines genetic drift is inconsistent with the genetic measurements. The same is true for the hypothesis that demography together with heritability of female RS (as measured here) are the only contributing factors. The modulation of polygyny on demography is shown to be a strong factor, as only when assuming polygyny of 8%-11% (point estimate 10.6%) mating males per generation, or 12%-22% (point estimate 19.5%) mating males accounting also for heritability of female RS, can the observed genetic data be explained by the hypotheses raised (Fig.1 and Fig.S1). The higher range compares with other strong polygynous mating-systems documented in mammals, such as Antarctic fur seal (Hoffman et al. 2003), fallow deer (Say et al. 2003), three-toed sloth (Pauli et al. 2012), and elephant seals (Hoelzel et al. 1999). However, the lower range is more extreme than any of these strongly-polygynous systems. It is therefore more likely that female heritability in RS contributes considerably to genetic drift and polygyny is at the level closer to 20% mating males per generation, but not as extreme as 10%. Nevertheless, the effects of extreme modulation of polygyny on demography without heritability of female RS are also consistent with the genetic data (Fig.1 and Fig.S1).

Heritability of RS is rarely documented in wild mammals ((Charmantier et al. 2014); see (Kelly 2001) and (Blomquist 2009) for examples of such studies), since analyzing it requires long-term monitoring over at least three generations, and individual-level recognition of at least the first two generations. Here we found female RS to be highly heritable ($h_f^2 = 0.91$), which implies that female RS is mainly inherited maternally. This suggests that heritability is not only due to genetically inherited traits, as it is unlikely that the large number of traits influencing RS will be mostly inherited maternally. On the other hand, social inheritance, if significant enough, could be a plausible explanation for such heritability, since Asiatic wild ass are social animals, and daughters most often remain with their mother at least until the age of three and have no association with their father. If indeed social inheritance is the main source of heritability of RS in the population, then the assumption of $h_m^2 \approx 0$ is justified due to lack of social association between fathers and sons; otherwise, h_m^2 could be

approximated in future studies, for example by paternity analysis, and paternal inheritance can be incorporated as an additional N_{ev} prediction.

Heritability of RS through social inheritance could be mediated by inheritance of social status and/or by transfer of knowledge (e.g. distribution of key resources) from parent to offspring, as both mechanisms are often linked to RS. For example, heritability of fitness in cheetahs (Kelly 2001) may also be related to social inheritance (Nomura 2002). While inheritance of social status and the relation between social status and RS have not been studied in wild ass, heritability of social status does occur in Equids (Haupt & Wolski 1980; Feh 1999) and other social mammals (Holekamp & Smale 1991; Pereira 1995; Engh et al. 2000; Ilany & Akçay 2016). Social learning is the focus of many studies (Galef & Laland 2005), and has been observed in wild asses (Rubenstein et al. 2015), but there is little information regarding parent-offspring information transfer in this species. Nevertheless, the non-uniform distribution of resources in the arid environment of the Negev may potentially allow for parent-offspring knowledge transfer to have a considerable effect on heritability of RS (Danchin 2004).

Although this study focused on variance effective size, N_{ev} , some inference regarding inbreeding effective size, N_{ef} , can be made. While direct estimation of N_{ef} would require detailed pedigree data or genetic parentage analysis of the population over the entire time period considered, in populations experiencing demographic growth N_{ef} tends to be smaller than N_{ev} (Templeton 2006). For example, the demographic inbreeding effective size given the data in Table 2 is $\overline{N_d} = 52.4$ (51.4-53.2), less than half the demographic variance effective size (the harmonic mean of census numbers in generations 0-3; (Templeton 2006)). While different aspects of life-history and mating-system may affect N_{ef} and N_{ev} in different ways, it is very likely that N_{ef} is lower than N_{ev} in this expanding population.

Conservation implications

Although the Asiatic wild ass population shows positive population growth, the low N_{ev} , and by implication the low N_{ef} , are of conservation concern. By separating the impacts of life-history and mating-system on genetic drift to different factors, we gain detailed information that can now be used to inform managers and help design management protocols for increasing effective population sizes. As the population grows and approaches carrying capacity, N_d should increase and with it N_{ev} . However, the other factors, related more to behavioral and ecological characteristics of the species, are not expected to change without intervention. Variance in female lTRS does not seem to be a significant contributor to genetic drift, and is therefore less of an issue in this population.

The main factor contributing to genetic drift in this system is polygyny, with a reduction of 81% in N_{ev} with $p_0 = 0.106$ ($N_p = 0.19N$; eq. 2 assuming sex-ratio 1:1) or 67% with $p_0 = 0.195$ ($N_p = 0.33N$). In this species, the proportion of mating males, p_0 , is determined mainly by the availability of territories, since male wild ass roam in bachelor groups until they can attain and defend a valuable territory, thus gaining access to mating opportunities (Saltz et al. 2000). In the Negev desert, the population is centered around the few artificial and natural water-sources in the region (Nezer et al. 2016). GPS data suggest that territories are formed mainly around these water sources, and occupied mostly in summer, the breeding season (Giotto et al. 2015). Thus, p_0 is probably constrained by the availability of water sources, and more water sources may allow for more territories to be formed, consequently allowing for more males to attain territories and gain access to mating opportunities.

Increasing p_0 in this fission-fusion population would be similar to changing harem size in populations with a harem-polygyny social structure. However, in the case of the wild ass in the Negev, this manipulation is made easier by the fact that territories are formed around water sources. Currently, artificial water-source supply is aimed only at population sustainability, but considering the strong

effect of polygyny on N_{ev} , we would recommend placing more artificial water sources to increase p_0 , which should lead to increase in effective population sizes.

Maternal inheritance of ItRS also has considerable impact on genetic drift, and may be responsible for a 41% reduction in N_{ev} ($N_h = 0.59N$). This characteristic may, as mentioned above, have either or both genetic and social components. While intervention in genetic inheritance and social-status inheritance is difficult and probably inadvisable, parent-offspring transfer of knowledge regarding key resources may be affected by the distribution of these resources. Specifically, if the uniformity in which important resources are spatially distributed is increased, the effect of knowledge transfer, and hence heritability of RS (to the extent it is determined by social learning), should be decreased. Therefore, adding artificial water sources, as suggested above, may increase effective population sizes both by increasing p_0 and by decreasing h_f^2 .

Conclusion

By examining the consistency of different hypothesized life-history effects with empirical genetic measurements of N_{ev} , a comprehensive account of life-history and mating-system can be attained. Moreover, understanding the relative impacts of different life-history traits on effective population size in the context of genetic measurements can help focus management efforts on the factors most affecting genetic drift. This can complement sensitivity and elasticity analyses, which evaluate relative impact of model parameters on effective size (e.g. (Campbell & Husband 2005)), by allowing the comparison of many different models in relation to actual genetic estimates. This approach emphasizes the importance of having both genetic data and detailed demographic records of wild populations, as they can be used together to enrich our ecological understanding of populations.

The strategy we present is not at all limited to the hypotheses we chose to focus on. Effective population sizes can be predicted based on various aspects of demography, life-history, ecology and even population structure (to the extent that they can be simplified to tractable models). For different populations, different data may be available, and different questions may be of conservation interest. Nevertheless, whenever genetic estimates can be attained and plausible hypotheses can be made, estimation and predictions of effective sizes can be compared to inform conservation, as demonstrated here.

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Allendorf FW, Luikart GH, Aitken SN. 2012. Conservation and the genetics of populations. Wiley-Blackwell, West Sussex, UK.

Blomquist GE. 2009. Fitness-related patterns of genetic variation in rhesus macaques. *Genetica* **135**:209–219.

Campbell LG, Husband BC. 2005. Impact of clonal growth on effective population size in *Hymenoxys herbacea* (Asteraceae). *Heredity* **94**:526–532.

Charmantier A, Garant DD, Kruuk LEB. 2014. Quantitative genetics in the wild. Oxford University Press, Oxford, UK.

Danchin E. 2004. Public Information: From Nosy Neighbors to Cultural Evolution. *Science* **305**:487–491.

This article is protected by copyright. All rights reserved.

- Engh A, Esch K, Smale L, Holekamp K. 2000. Mechanisms of maternal rank “inheritance” in the spotted hyaena, *Crocuta crocuta*. *Animal behaviour* **60**:323–332.
- Feh C. 1999. Alliances and reproductive success om Camargue stallions. *Animal Behaviour* **57**:705–713.
- Galef B, Laland KN. 2005. Social Learning in Animals: Empirical Studies and Theoretical Models. *BioScience* **55**:489.
- Giotto N, Gerard JF, Ziv A, Bouskila A, Bar-David S. 2015. Space-Use Patterns of the Asiatic Wild Ass (*Equus hemionus*): Complementary Insights from Displacement, Recursion Movement and Habitat Selection Analyses. *PLoS ONE* **10**:e0143279.
- Gueta T, Templeton AR, Bar-David S. 2014. Development of genetic structure in a heterogeneous landscape over a short time frame: the reintroduced Asiatic wild ass. *Conservation Genetics* **15**:1231–1242.
- Hoelzel AR, Le Boeuf BJ, Reiter J, Campagna C. 1999. Alpha-male paternity in elephant seals. *Behavioral Ecology and Sociobiology* **46**:298–306.
- Hoffman JI, Boyd IL, Amos W. 2003. Male reproductive strategy and the importance of maternal status in the antarctic fur seal *Arctocephalus gazella*. *Evolution* **57**:1917–1930.
- Holekamp KE, Smale L. 1991. Dominance acquisition during mammalian social development: The “inheritance” of maternal rank. *Integrative and Comparative Biology* **31**:306–317.
- Haupt K a, Wolski TR. 1980. Stability of equine hierarchies and the prevention of dominance related aggression. *Equine veterinary journal* **12**:15–8.
- Ilany A, Akçay E. 2016. Social inheritance can explain the structure of animal social networks. *Nature Communications* **7**:12084.

Kaczensky P, Lkhagvasuren, B. Pereladova O, Hemami M, Bouskila A. 2015. *Equus hemionus*. The IUCN Red List of Threatened Species:e.T7951A45171204.

Kelly MJ. 2001. Lineage Loss in Serengeti Cheetahs: Consequences of High Reproductive Variance and Heritability of Fitness on Effective Population Size. *Conservation Biology* **15**:137–147.

Leberg PL. 2005. Genetic approaches for estimating the effective size of populations. *Journal of Wildlife Management* **69**:1385–1399.

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW. 2010. Estimation of census and effective population sizes: The increasing usefulness of DNA-based approaches. *Conservation Genetics* **11**:355–373.

Nei M, Murata M. 1966. Effective population size when fertility is inherited. *Genetics Research* **8**:257–260.

Nei M, Tajima F. 1981. Genetic drift and estimation of effective population size. *Genetics* **98**:625–640.

Nezer O, Bar-David S, Gueta T, Carmel Y. 2016. High-resolution species-distribution model based on systematic sampling and indirect observations. *Biodiversity and Conservation* **26**:421–437.

Nomura T. 2002. Effective size of populations with heritable variation in fitness. *Heredity* **89**:413–6.

Nunney L. 1996. The influence of variation in female fecundity on effective population size. *Biological Journal of the Linnean Society* **59**:411–425.

Pauli JN, Peery MZ, Festa-Bianchet M. 2012. Unexpected Strong Polygyny in the Brown-Throated Three-Toed Sloth. *PLoS ONE* **7**:e51389.

Pereira ME. 1995. Development and social dominance among group-living primates. *American Journal of Primatology* **37**:143–175.

This article is protected by copyright. All rights reserved.

Renan S, Greenbaum G, Shahar N, Templeton AR, Bouskila A, Bar-David S. 2015. Stochastic modeling of shifts in allele frequencies reveals a strongly polygynous mating system in the reintroduced Asiatic wild ass. *Molecular Ecology* **24**:1433–1466.

Robertson A. 1961. Inbreeding in artificial selection programmes. *Genetics Research* **2**:189–194.

Rubenstein DI. 1994. The Ecology of Female Social Behaviour in Horses, Zebras and Asses. Pages 13–28 in P. Jarman and A. Rossiter, editors. *Animal Societies: Individuals, Interactions, and Organization*.

Rubenstein DI, Sundaresan SR, Fischhoff IR, Tantipathananandh C, Berger-Wolf TY. 2015. Similar but different: Dynamic social network analysis highlights fundamental differences between the fission-fusion societies of two equid species, the onager and Grevy's zebra. *PLoS ONE* **10**:e0138645.

Saltz D, Rowen M, Rubenstein DI. 2000. The effect of space-use patterns of reintroduced asiatic wild ass on effective population size. *Conservation Biology* **14**:1852–1861.

Saltz D, Rubenstein DI. 1995. Population dynamics of a reintroduced Asiatic wild ass (*Equus hemionus*) herd. *Ecological Applications* **5**:327–335. Ecological Society of America.

Saltz D, Rubenstein DI, White GC. 2006. The impact of increased environmental stochasticity due to climate change on the dynamics of asiatic wild ass. *Conservation Biology* **20**:1402–1409.

Say L, Naulty F, Hayden TJ. 2003. Genetic and behavioural estimates of reproductive skew in male fallow deer. *Molecular Ecology* **12**:2793–2800.

Schwartz MK, Tallmon DA, Luikart G. 1998. Review of DNA-based census and effective population size estimators. *Animal Conservation* **1**:293–299.

Templeton AR. 2006. *Population genetics and microevolutionary theory*. John Wiley & Sons, Hoboken, New Jersey.

Wade MJ, Shuster SM. 2004. Sexual selection: harem size and the variance in male reproductive

This article is protected by copyright. All rights reserved.

success. *The American naturalist* **164**:E83-9.

Wang J. 2001. A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genetics Research* **78**:243–257.

Wang J. 2005. Estimation of effective population sizes from data on genetic markers. *Philosophical transactions of the Royal Society B* **360**:1395–1409.

Wang J, Hill WG. 2000. Marker-assisted selection to increase effective population size by reducing Mendelian segregation variance. *Genetics* **154**:475–489.

Wang J, Santiago E, Caballero A. 2016. Prediction and estimation of effective population size. *Heredity* **117**:1–14.

Waples RS, Yokota M. 2007. Temporal estimates of effective population size in species with overlapping generations. *Genetics* **175**:219–33.

Wright S. 1931. Evolution in Mendelian populations. *Genetics* **16**:97–159.

Tables

Table 1 – Parameters and notations used in the manuscript, with remarks regarding how each parameter was evaluated.

Parameter/notation	Definition	Remarks
N_{ev}	Variance effective population size	
N_{ef}	Inbreeding effective population size	
N_m, N_f	Census number of males and females, respectively	Evaluated from demographic data (Table 2)
N_d	Per-generation demographic (census) variance effective population size	Evaluated from demographic data (Table 2)
$\overline{N_d}$	Overall (multi-generational) demographic variance effective population size	
p_0	Proportion of mating males in the population	
N_p	Per-generation variance effective population size accounting for demography and polygyny	We considered the range $0 \leq p_0 \leq 0.25$
$\overline{N_p}$	Overall (multi-generational) variance effective population size accounting for demography and polygyny	
v_m, v_f	Male and female variance in life-time reproductive success, respectively	For males assumed to be Poisson-distributed among mating males, for females evaluated from observations
N_r	The effect of variance reproductive success on the variance effective population size (with regards to ideal size N)	
h_m^2, h_f^2	Male and female heritability in life-time reproductive success, respectively	For males, assumed to be $h_m^2 = 0$; for females h_f^2 was evaluated from mother—daughter regression (Fig. 2)
N_h	The effect of heritability of reproductive success on the variance effective population size (with regards to ideal size N)	

Table 2 - Allele frequencies during the reintroduction period and in 2012 for 8 microsatellite loci. In parenthesis are sample sizes (reintroduction/2012).

	Reintroduction							2012						
	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7
HMS2 (28/113)	0.55	0.27	0.13	0.05				0.43	0.21	0.03	0.33			
HMS3 (28/125)	0.43	0.21	0.18	0.18				0.2	0.38	0.35	0.07			
HMS6 (30/127)	0.75	0.25						0.81	0.19					
AHT4 (27/113)	0.35	0.2	0.17	0.08	0.07	0.07	0.06	0.08	0.2	0.48	0	0.11	0	0.13
HTG4 (28/109)	0.54	0.43	0.03					0.57	0.42	0.01				
LEX74 (29/123)	0.43	0.43	0.14					0.55	0.4	0.05				
COR70 (26/88)	0.33	0.23	0.23	0.21				0.16	0.18	0.09	0.16			
UM11 (27/120)	0.94	0.06						0.81	0.19					

Table 3 - Estimated census sizes from direct counts and life-history table and demographic-predicted variance effective size (N_d) for each generation. In parenthesis are estimations with error margins of $\pm 10\%$ of the R_0 .

	Reintroduction (0)	Generation 1	Generation 2	Generation 3	Generation 4
Males (N_m)	12	33	56*	105*	196*
Females (N_f)	10	27	56*	105*	196*
N_d	21.8	59.4	112* (101-123)	210* (189-231)	392^* (353-432)

* Estimated assuming $R_0 = 1.87$.

Table 4 - Annual reproductive success of female Asiatic wild ass, mothers and daughters, during the years 1979-1996; RY (reproductive years) = number of years above age 3 during the study period; aRS = annual reproductive success; ItRS=estimated life-time reproductive success. Individuals may appear both in the mothers column and in the daughters column.

Mother	RY	Offspring	aRS	ItRS	Daughter	RY	Offspring	aRS	ItRS	Mean daughter aRS
Aphrodita	10	4	0.4	4.24	Annie	6	5	0.83	8.83	0.58
					Apalusa	3	1	0.33	3.53	
Alison	8	3	0.38	3.98	Alia*	2	0	-	-	0
					Ayala	5	0	0	0	
Crookedtail	12	8	0.67	7.07	Clairec	6	4	0.67	7.07	0.5
					Cleo	3	1	0.33	3.53	
					Daphodile*	2	0	-	-	
Daphne	8	7	0.88	9.28	Daisy*	1	0	-	-	0.67
					Dalia	6	4	0.67	7.07	
					Daphne	8	7	0.88	9.28	
Dart	12	11	0.92	9.75	Drea*	2	0	-	-	0.88
					Haily*	1	0	-	-	
Hannah	10	9	0.9	9.54	Heather	7	6	0.86	9.09	0.86
					Heidi	4	2	0.5	4.06	
Radio	12	9	0.75	7.95	Rhapsody*	1	0	-	-	0.47
					Radner*	2	0	-	-	
					Rachel	3	1	0.33	3.53	
					Roxan	5	3	0.6	6.36	
					Rita	4	2	0.5	5.3	
Rhonda	12	8	0.67	7.07	Rose	3	1	0.33	3.53	0.47
					Sealy*	0	0	-	-	
Seven	12	7	0.58	4.89	Seneca*	2	0	-	-	0
					Tanya	4	2	0.5	5.3	
Theresa	12	8	0.67	7.07	Thelma*	2	0	-	-	0.5

* Data regarding RS of females with less than 3 reproductive years during the study were not included in analyses.

Figure legends

Figure 1 - Demographic and life-history impacts on variance effective population size (N_{ev}). In purple is the estimated N_{ev} from genetic data using the Maximum Pseudo-Likelihood method. Predictions of N_{ev} under different hypothesis of contributing factors to genetic drift appear in different colors: in green only demography ($\overline{N_d}$); in red demography and inheritance of female ItRS ($\overline{N_d}$ and N_h); in blue demography and polygyny ($\overline{N_p}$); in orange demography, polygyny and inheritance of female ItRS ($\overline{N_p}$ and N_h). Shaded areas indicate margins of error. Horizontal lines indicate point estimates of p_0 , dashed lines indicate the ranges considering estimated errors.

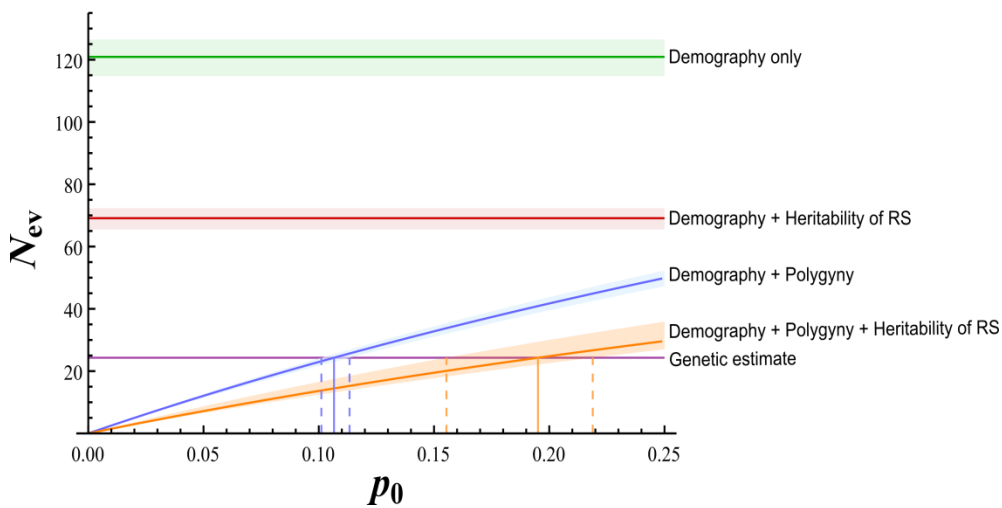


Figure 2 — Mother/mean-daughter regression of annual reproductive success (aRS).

