

The effects of cyclic dynamics and mating system on the effective size of an island mouflon population

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Abstract

The Haute Island mouflon (*Ovis aries*) population is isolated on one small (6.5 km²) island of the remote Kerguelen archipelago. Given a promiscuous mating system, a cyclic demography and a strong female-biased sex ratio after population crashes, we expected a low effective population size (N_e). We estimated N_e using demographic and temporal genetic approaches based on genetic information at 25 microsatellite loci from 62 and 58 mouflons sampled in 1988 and 2003, respectively. Genetic N_e estimates were higher than expected, varying between 104 and 250 depending on the methods used. Both demographic and genetic approaches show the Haute Island N_e is buffered against population crashes. The unexpectedly high N_e likely results from the cyclic winter crashes that allow young males to reproduce, limiting the variance of male reproductive success. Based on individual-based simulations, we suggest that despite a strongly female-biased sex ratio, the effects of the mating system on the effective population size more closely resemble random mating or weak polygyny.

Keywords: demographic methods, effective population size, genetic methods, mating system, mouflon, population dynamics

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Introduction

Effective population size (N_e), the size of an ideal population that would have the same rate of loss of genetic diversity as the studied population, is a measure largely used to quantify the loss of genetic diversity. 'Ideal' here includes several features, such as random mating, balanced sex ratio, nonoverlapping generations and an equal probability for all adults to contribute offspring to the next generation (Wright 1931, 1938; Kimura & Crow 1963; Caballero 1994). A small effective population size predicts a rapid loss of genetic diversity caused by genetic drift (Frankham *et al.* 2002). This loss is assumed to increase the genetic load, limit the evolutionary potential, and increase the risk of population extinction (Newman & Pilson 1997; Spielman *et al.* 2004).

Because N_e is strongly related to population size, the dynamics of the population is known to have a strong influence on N_e . For example, N_e is generally lower in fluctuating populations than in stable populations (Wright 1938; Vucetich *et al.* 1997). The mating system also strongly affects the dynamics of genetic diversity (Wright 1938; Nunney 1991, 1993; Caballero 1994); N_e should be lower for populations characterized by a large variance in reproductive success among individuals (i.e. promiscuous or polygynous populations) than for populations with a low interindividual variance in reproductive success (i.e. monogamous populations).

The effects of population dynamics and size (Nei *et al.* 1975; Motro & Thomson 1982; Vucetich *et al.* 1997; Montgomery *et al.* 2000), or mating system (Kaeuffer *et al.* 2004; Matocq 2004) on N_e have largely been investigated separately, and their combined impact on N_e is not well understood and difficult to predict. For example, in Soay sheep on St Kilda, a population characterized by a polygynous mating system

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and by fluctuating population density, when density is low or the sex ratio strongly biased in favour of females the decrease in intermale reproductive competition allows juvenile males to achieve reproductive success (Pemberton *et al.* 1996, 1999). Coltman *et al.* (1999) suggested that this situation would reduce the between-male variance in reproductive success, which in turn would limit the potential negative impact of mating system and population fluctuation on N_e .

Here, we use the well-documented history of an insular population, the Kerguelen mouflon (*Ovis aries*) population, to investigate the combined effect of mating system and population dynamics on N_e . In 1957, one male and one female mouflon lambs were introduced on a small island of the Kerguelen archipelago (Southern Indian Ocean, Chapuis *et al.* 1994). The two individuals started reproducing 1 year after their introduction, and the population grew rapidly during the subsequent 20 years. After reaching a size of 700 individuals, the population entered a cycling phase, with fluctuations of large amplitude and frequent winter crashes affecting males more than females (Boussès *et al.* 1994; Chapuis *et al.* 1994). Given the cyclic demography of the population, the promiscuous mating system of mouflons based on intense male–male competition for the access to oestrus females (Bon *et al.* 1992), and the highly female-biased sex ratio following winter crashes, we expected a small N_e , and thus, a high risk of extinction. Forty-six years after its introduction, however, the Kerguelen mouflon population persists with several hundred individuals.

In this study, we compare estimates of effective population size obtained with different demographic and genetic models of the Kerguelen mouflon population. The estimation of N_e based on demographic information (i.e. population size, sex-ratio) allowed us to estimate the expected loss of genetic diversity given the demographic parameters observed (Caballero 1994). The genetic approach uses information on changes in allele frequencies to estimate the rate of drift in the population (Waples 1989). This method reflects the observed effective size of the population, but does not provide insight into the causal factors underlying a particular value of N_e . By combining both methods, one can determine which factors are central to the dynamics of the genetic diversity of a given population. To understand the combined effects of population dynamics and mating system on N_e in the Kerguelen mouflon population, we use an individual-based simulation approach under a neutral genetic model (assuming no selection, no mutation, and no population structure).

Material and methods

Study population

The population is located on Haute Island, a small island (6.5 km²) of the Kerguelen archipelago. Kerguelen is a very

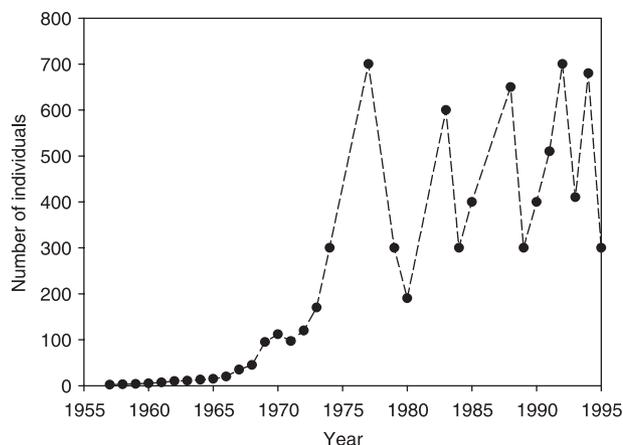


Fig. 1 Number of individuals estimated for the Kerguelen mouflon population.

remote sub-Antarctic archipelago located in the Southern Indian Ocean (49°20'S, 70°20'E). The climate is sub-Antarctic, with high precipitation, strong winds and average temperature ranging from 2 °C during the winter to 8 °C during summer. Rocky landscapes dominate Haute Island, with sparse vegetation cover (about 40%) composed of a few endemic species (i.e. *Azorella selago*, *Agrostis magellanica*) and introduced forage species (i.e. *Poa annua* and *Dactylis glomerata* Chapuis *et al.* 1994).

The two founder individuals that arrived in 1957 originated from the Vincennes Zoo (Paris, France). The population reached 100 individuals at the beginning of the 1970s. The population then grew exponentially, reaching about 700 individuals in 1977, which corresponds to a density of 100 individuals per square kilometre. Since then the population has been characterized by cyclical dynamics, fluctuating between 250 and 650 individuals, with winter crashes occurring at a periodicity of 3–5 years (Chapuis *et al.* 1994) (Fig. 1). Winter crashes occurred after the population exceeded about 650 individuals. Males are affected by a crash more strongly than females because of the strong reproductive effort related to intermale competition that occurs during the rut, just before winter (Boussès *et al.* 1994). This situation is similar to the Soay Sheep population, where the effect of population dynamics on individual reproductive success is well known (i.e. Coltman *et al.* 1999; Pemberton *et al.* 1999).

Three monthly surveys of the population were performed along a standard transect between 1989 and 1995. We used surveys carried out during the austral summer (a period of low mortality and that follows the birth season) to estimate the number of mouflon in each sex and age class (i.e. three categories: lambs, yearlings and adults ≥ 2 years). The sex ratio (number of adult males/number of adults) was always female biased and varied from 0.02 to 0.37 from year to year (Table 1; Boussès *et al.* 1994; Réale 1996).

Table 1 Effective number of breeders and Nb/N ratio estimated between 1989 and 1995 in the Haute Island mouflon population. Nb_t is the effective number of breeders at year t estimated as $Nb_t = 4 N_m N_f / N_t$, where N_m and N_f are the number of males and females, respectively, and $N_t = N_m + N_f$ at year t . α represents the factor of reduction of Nb caused by an unbalanced sex ratio, φ the factor of reduction of Nb caused by population fluctuations, and β the cumulative effects of an unbalanced sex ratio and fluctuating population size (Kalinowski & Waples 2002)

Year	Adults					Including yearlings						
	N_m	N_f	N_t	N_m/N_t	Nb_t	Nb_t/N_t	N_m	N_f	N_t	N_m/N_t	Nb_t	Nb_t/N_t
1989	3	133	136	0.02	12	0.09	14	141	155	0.09	51	0.33
1990	60	181	242	0.25	181	0.75	67	196	263	0.25	200	0.76
1991	47	181	228	0.21	149	0.66	127	230	356	0.36	327	0.92
1992	156	263	419	0.37	392	0.94	222	309	531	0.42	517	0.97
1993	46	225	271	0.17	153	0.56	49	232	282	0.18	163	0.58
1994	60	277	338	0.18	198	0.59	139	314	453	0.31	386	0.85
1995	30	228	258	0.11	105	0.41	31	235	265	0.12	109	0.41
Nb					58						147	
$\alpha = Nb/N_{Harm}$						0.24						0.51
$\varphi = N_{Harm}/N_{Arit}$						0.9						0.87
$\beta = Nb/N_{Arit}$						0.21						0.45

Genetic analysis

Seven lambs (5 male, 2 females), eight yearlings (4 males, 4 females) and 47 adults (31 males and 16 females; total = 62 individuals) were shot in 1988. Eight lambs (1 male, 4 females, 3 undetermined), 11 yearlings (6 males, 5 females) and 39 adults (26 males and 13 females; total = 58 individuals) were shot in 2003. Tissue samples (i.e. ear punch) were collected on all the bodies and stored in 95% ethanol and DNA extraction was performed with the QIAamp tissue extraction mini kit (QIAGEN Inc.), following the instructions provided with the kits.

Genotypes were determined at 25 microsatellite loci amplified by polymerase chain reaction (PCR): ARO28, HEL10, MCM64, MCM152, BM3413, BM848, HUI177, MAF64, MCM527, TGLA13, Ilts059, TGLA176, RT1, AGLA226, Il2ra, MCM218, NRAMP, OarCP49, TEXAN4, DRBps, INRA26, oMHC1, TGLA387, CSSM022 and MAF33. For more detailed microsatellite primer information, see Maddox *et al.* (2001) and <http://www.thearkdb.org/>. PCRs were then carried out in 10- μ L reaction mixtures containing 0.8 μ L of dNTPs (2 mM each), 0.2–0.4 μ L of $MgCl_2$ (50 mM), 0.08 μ L of forward and reverse primers (20 μ M), 1 μ L of 10 \times buffer, 0.1 μ L of BSA (30 mg/mL), 0.05 U/ μ L *Taq* polymerase (Bioline), and 1 μ L of the DNA extract (10 ng/ μ L). The thermal profile consisted of an initial DNA denaturation of 3 min at 95 $^{\circ}C$, followed by 44 cycles of 30-s DNA denaturation at 94 $^{\circ}C$, 30-s primer annealing at 49–62 $^{\circ}C$, and 30-s DNA extension at 72 $^{\circ}C$. To conclude PCR, the temperature was then increased to 72 $^{\circ}C$ for 10 min, and then to 10 $^{\circ}C$ during 10 min. PCR products were visualized on an automated sequencer (ABI PRISM 3730, Applied Biosystems) and analysed using the software GENEMAPPER (Applied Biosystems). For each locus,

we tested for deviation from Hardy–Weinberg equilibrium by exact tests (Guo & Thompson 1992) using GENEPOP software (Raymond & Rousset 1995). A sequential Bonferroni correction for multiple tests was applied (Rice 1989). We also estimated F_{IS} (Weir & Cockerham 1984) using GENEPOP, and the observed and expected heterozygosities using GENETIX software (Belkhir *et al.* 2000). Deviation from Hardy–Weinberg equilibrium and a large F_{IS} can indicate the presence of null alleles. Furthermore, to assess the reliability of our genotyping, all of the samples were genotyped twice for 15 randomly chosen loci.

Effective population size

Demographic method Lifetime variance in reproductive success is necessary to estimate effective population size. However, individual life-history information was lacking in the Kerguelen population. Therefore, based on the number of individuals in each sex and age class obtained from the monthly surveys over the period 1989–1995, we estimated the annual effective number of breeders following the formulae:

$$Nb_t = \frac{4NmNf}{Nm + Nf} \quad (1)$$

(Falconer & Mackay 1996) where Nb_t is the effective number of breeders at year t and Nm and Nf are the number of adult males and females at year t , respectively. We then estimated the demographic Nb as the harmonic mean of Nb_t values (e.g. Hartl & Clark 1997). We used Nb , estimated over two to three generations of mouflons (i.e. between 1989 and 1995), as a proxy for the demographic N_e .

Following Kalinowski & Waples (2002), we then estimated the cumulative effect of the unbalanced sex ratio on the Kerguelen mouflon effective number of breeders between 1989 and 1995 by calculating $\alpha_{A'}$, the ratio of our estimate of Nb to N_{Harm} , the harmonic mean adult population size. We also estimated the effect of population fluctuations on Nb by calculating $\varphi_{A'}$, the ratio of N_{Harm} on N_{Arit} , the arithmetic mean population size. Finally, we estimated the combined effect of an unbalanced sex ratio and of population fluctuation on the reduction of effective number of breeders, by calculating the ratio $\beta_A = Nb/N_{\text{Arit}}$. In contrast with other wild ovid populations (Bon *et al.* 1992; Coltman *et al.* 2002), yearlings appeared to be involved in reproduction in the Haute Island population (Réale 1996; Boussès & Réale 1998). Thus we also provide estimates of α_Y , φ_Y and β_Y including yearlings. Although our estimation covers only a period of 7 years, Kalinowski & Waples (2002) have shown that α , φ and β can quickly reach an asymptotic value and can be representative of a long-term effective breeders' population size when the sampling period is representative of the dynamics of the population.

Genetic methods N_e was estimated from the change in allele frequencies between 1988 and 2003 using Waples' (1989) moment estimator method and two recent temporal likelihood estimators proposed by Berthier *et al.* (2002) and by Wang (2001). For each of these three methods, we used the allele frequencies measured at the 25 microsatellite loci.

Waples' (1989) method is based on changes in allele frequencies (e.g. F) between two samples separated by t generations. F is inversely proportional to effective population size (Caballero 1994). We estimated the standardized variance of the change in allele frequencies F_k , for each locus using the equation given by Pollak (1983):

$$F_k = \frac{1}{K-1} \sum_{i=1}^K \frac{(x_i - y_i)^2}{\left(\frac{x_i + y_i}{2}\right)} \quad (2)$$

where K is the number of alleles, and i is the frequency of a given allele at times where the first (x) and second sample (y) were collected. F_k was then averaged over all loci and weighted by the number of independent alleles at each locus. Our samples were taken without replacement (see Waples 1989). Some individuals, however, could have reproduced before being sampled therefore the effective population size was estimated following Waples (1989) equation 12:

$$\hat{N}_e = \frac{t}{2[F_k - 1/(2S_0) - 1/(2S_t) + 1/N]} \quad (3)$$

where S_0 and S_t are sample sizes at time 0 and t , respectively, N is total number of individuals in the population at time 0 and t is the number of generations between the two

samples. Ninety-five per cent confidence intervals were estimated following Waples (1989).

Temporal methods generally assume discrete generations (Waples 1989). When applied to species with overlapping generations, these methods could bias estimates of N_e , especially in the case of sampling bias towards a specific age class or cohort (Jorde & Ryman 1994; Hauser *et al.* 2002). A method using demographic information such as age-specific survival and birth rate from each cohort has been therefore proposed by Jorde & Ryman (1995). In the absence of appropriate demographic information, we could not use this method. The large (> 4) number of generations between our two samples should limit the potential bias caused by overlapping generations (see Miller & Kapuscinski 1997; Hauser *et al.* 2002; Waples & Yokota 2007).

Wang's (2001) method is based on the idea that N_e can be estimated by maximum likelihood, given the allelic frequencies observed at different times (Williamson & Slatkin 1999). Wang's method performs similarly to Williamson & Slatkin's model (1999), although with less computation (i.e. MLNE software; Wang 2001). Wang's method requires a fixed maximum N_e value. We fixed this value to 1000 individuals.

The estimator proposed by Berthier *et al.* (2002) uses the genealogical information rather than the change in gene frequencies. This method includes a Bayesian prior of the maximum N_e of the population (i.e. TM3 software; Berthier *et al.* 2002) which computes the maximum-likelihood value as well as the 5% and 95% quantiles of the posterior distribution of N_e . We fixed the prior to 1000 individuals, and 50 000 replicates were made for each generation time.

The two likelihood methods (i.e. Berthier *et al.* 2002 and Wang 2001) are expected to give more precise results (i.e. smallest CI) than Waples' method (Wang 2001; Berthier 2002; Tallmon *et al.* 2004; but see Kaeuffer *et al.* 2004; Poulsen *et al.* 2006). However, in contrast to Waples' method (Waples 1989 and see above, equation 3), both Wang's and Berthier's methods do not incorporate a correction in sampling caused by individuals who reproduced before being sampled (for more general discussion see also Waples & Yokota 2007).

A central parameter in the three genetic methods is the number of generations between samples. Number of generations was estimated as the number of years between the two samples divided by the average generation time. In ovids, the age of each individual can be estimated precisely for males by using the number of horn segments (Geist 1971), and can be approximated for females by using the number and wear of definite incisors (Boussès & Réale 1994). In European populations, mouflon life span can reach up to 13 years (Bon *et al.* 1992), and therefore a generation time of about 5 years is generally expected for this species. However, on Haute Island, only five males out of 388 exceeded the age of 5 years between 1993 and 1995, a period of

intensive study of the population. Lamb males have been observed involved in the rutting groups (D. Réale, personal observation), and a significant portion of females could get fertilized at 18 months of age (Boussès & Réale 1998). In the absence of precise information on the average age at reproduction, we therefore used three different plausible values of generation time (2, 3 and 4 years).

Simulation

The individual-based approach simulated the demography and the genealogy of a population characterized by demographic parameters similar to those observed in the Kerguelen mouflon population. We considered the following parameters: mating system, female fecundity, variance in fecundity among females, juvenile mortality rate, adult lifespan, carrying capacity and mortality rate during a population crash as soon as the population size exceeds the carrying capacity of the environment (see below). The model considered a diploid population similar to the Kerguelen population, with an initial size of two (the founder individuals) at the initial time $t = 0$, and no selection, mutation, or population structure. The genotypes of the two founder individuals (one male and one female) were constructed using the number of alleles (from two to four) observed at each of the genotyped loci (see above). At each new generation, individuals were randomly paired to produce offspring. The genotype of each individual was constructed by randomly selecting one allele at each of the 25 loci from each of the two parents. The gender of an individual was randomly assigned at birth, with a 0.5 probability for each sex.

Mouflons have a promiscuous mating system (Bon *et al.* 1992), but we do not know the proportion of males involved in mating on Haute Island. On one hand, competition might be restricted and mating could be considered as random. On the other hand, strong competition between males may limit the access to oestrus females to only a few males. We therefore investigated the effect of random and polygynous mating systems on the estimation of N_e . In the random mating system, all individuals reproduced, and males and females were paired randomly. In the polygynous mating system, each year only a limited number of randomly chosen males reproduced with several females each, and all females reproduced. For the polygynous mating system, we used three different categories of proportions of males involved in mating (80%, 50% and 20%). In our simulations, reproducers were randomly chosen from the entire male (age ≥ 1) population. Thus, a given male could reproduce in two successive years just by chance. This mechanism could limit the variance in male lifetime reproductive success, especially in a polygynous mating system, and limit the decrease in N_e (Nunney 1993). Female fecundity was fixed to 1.1 (SD = 0.7), and was estimated

from the number of embryos per female counted in 69 culled females during winter 1994 (unpublished results). Based on demographic data (see Fig. 1; Boussès *et al.* 1994), we fixed the carrying capacity to 600 and the mortality rate during a crash to 70%.

To avoid adding too much complexity to the models and confounding factors in our results, we simulated populations with equivalent mortality rates in both sexes. This reduced the bias in sex ratio in favour of females in the population. Given that biased sex ratio plays a strong role on N_e (Nunney 1993), our simulated effective population size should be overestimated. The average adult lifespan estimated in the population is about 3.5 (median: 3; using the average age of adult carcasses found on the ground between 1993 and 1995; $N = 412$). To ensure that we did not underestimate lifespan, we used values of lifespan ranging from 3 to 5 years. We simulated populations with different combinations of lifespan and juvenile mortality rate (0.25–0.45) to explore the potential effects of these parameters on N_e . We selected these demographic parameters to mimic the demography observed in the population (i.e. maximum population size, number of peaks and crashes). Each simulation was run for a period of 47 years. Sixty individuals (age ≥ 1) were randomly chosen at years 32 and 47, to mimic the two samples collected in 1988 and 2003. From the genotypic information of the individuals sampled from the simulated populations, we calculated the variance of the change in allelic frequencies between the first and the second samples following Pollak (1983; see above). For each combination of demographic parameters (i.e. lifespan, juvenile mortality, and mating system), 1000 replicates were run to calculate the average value of the variance of the change in allelic frequencies. We then calculated \hat{N}_e using Waples' (1989) method.

We investigated the effect of initial heterozygosity on N_e by using either completely heterozygous ($H = 1$) founder individuals or the minimally heterozygous founder individuals, given the number of alleles per gene measured ($H = 0.22$) on Haute Island at the origin. Results using both scenarios were very similar, and only the scenario with $H = 1$ is shown here.

Results

Observed heterozygosity ranged from 0.18 to 0.73 (Table 2) and allelic diversity was low (two to four alleles). Only one locus (MCM64) nominally departed from Hardy–Weinberg equilibrium. After corrections for multiple tests, however, the observed heterozygosity did not differ significantly from that expected under Hardy–Weinberg assumptions. F_{IS} ranged between -0.101 and 0.181 . The error rate observed during replicate genotyping (new amplification and new scoring) of 15 loci was approximately 2–3% per genotype.

Table 2 Number of alleles per locus, expected (H_E) and observed (H_O) heterozygosities, F_{IS} and probability of departure (P) from Hardy–Weinberg equilibrium estimated for each locus (data for 1988 and 2003 were pooled together, but analyses with data separated gave similar results)

Loci	No. of alleles	H_E	H_O	P	F_{IS}
ARO28	2	0.36	0.31	0.2095	+0.127
HEL10	3	0.57	0.60	0.8141	-0.065
MCM64	3	0.64	0.57	0.0367	+0.105
MCM152	2	0.49	0.48	0.8511	+0.025
BM3413	2	0.49	0.47	0.6986	+0.046
BM848	2	0.44	0.42	0.6707	+0.056
HUJ177	2	0.47	0.38	0.0740	+0.181
MAF64	2	0.18	0.18	1	-0.015
MCM527	2	0.38	0.39	1	-0.021
TGLA13	4	0.67	0.72	0.0540	-0.049
Ilst059	2	0.42	0.39	0.4961	+0.082
TGLA176	3	0.62	0.64	0.6097	-0.029
RT1	2	0.19	0.19	0.6271	+0.053
AGLA226	3	0.65	0.60	0.2021	+0.087
Il2ra	2	0.48	0.48	1	+0.006
MCM218	3	0.51	0.56	0.6473	-0.101
NRAMP	2	0.38	0.35	0.3111	+0.104
OarCP49	3	0.55	0.53	0.8968	+0.035
TEXAN4	2	0.39	0.34	0.1114	+0.149
DRBps	2	0.50	0.49	0.8536	+0.022
INRA26	2	0.22	0.23	1	-0.057
oMHC1	3	0.56	0.52	0.1593	+0.074
TGLA387	2	0.49	0.56	0.1765	-0.140
CSSM022	2	0.45	0.48	0.5266	-0.077
MAF33	4	0.72	0.71	0.8355	+0.019

Table 3 Microsatellite locus name, and standardized variance in allele frequency change (F_{ki}), in the Haute Island mouflon population. F_{ki} is the value for each individual locus and F_k represents the average value over all the loci, respectively. F_{ki} and F_k are calculated following Pollak (1983)

Locus name	F_{ki}
ARO28	0.1106
HEL10	0.0197
MCM64	0.0287
MCM152	0.0413
BM3413	0.0542
BM848	0.0727
HUJ177	0.0163
MAF64	0.0061
MCM527	0.0004
TGLA13	0.0299
Ilst059	0.0001
TGLA176	0.0046
RT1	0.0072
AGLA226	0.0622
Il2ra	0.0491
MCM218	0.0194
NRAMP	0.0522
OarCP49	0.0143
TEXAN4	0.0133
DRBps	0.1807
INRA26	0.0034
oMHC1	0.0585
TGLA387	0.0049
CSSM022	0.0006
MAF33	0.0134
F_k	0.0332

The exponential growth of the Kerguelen mouflon population following its introduction allowed the effective population size to increase rapidly. Under the demographic conditions observed on Haute Island (i.e. biased sex ratio and fluctuating population), we estimated an Nb of 58 individuals when adults only were considered and an Nb of 147 when we included yearlings (Table 1). The unbalanced sex ratio reduced the effective number of breeders by 76% ($\alpha_A = 0.24$). Population fluctuations reduced Nb by 10% ($\varphi_A = 0.90$). When combined, these two factors reduced Nb by 79% ($\beta_A = 0.21$). When we included yearlings in the pool of reproductively active individuals, the unbalanced sex ratio reduced the effective number of breeders by 49% ($\alpha_y = 0.51$). Population fluctuation reduced the Nb by 13% ($\varphi_y = 0.87$), and their combined effect led to a reduction of Nb by 55% ($\beta_y = 0.45$).

The standardized variance of the change in allelic frequencies used in the Waples' method is given in Table 3. The three genetic methods produced similar estimates of \hat{N}_e (Fig. 2) ranging from 104 to 250, but Waples' estimates were the lowest. This difference may be partly attributable to the correction factor of $1/N$ used in the Waples' method

to account for reproduction of individuals before sampling. However, Waples' method without correcting factors still gives a \hat{N}_e estimate slightly lower than other genetic methods. \hat{N}_e decreased with the increase in generation time (Fig. 2). Waples' genetic methods provided \hat{N}_e/N ratios ranging from 0.38 to 0.77 (with $N = 270$ the total number of adults), depending on the generation time used (Table 4). Large confidence intervals were associated with each estimate, especially the likelihood-based estimators Berthier *et al.* (2002) and Wang (2001).

Simulations

Simulations gave variance in allelic frequencies and \hat{N}_e values consistent with the values of \hat{N}_e estimated using the genetic methods. \hat{N}_e declined with increasing generation time and increasing polygyny (Table 4). Increasing lifespan increased \hat{N}_e . For a given number of generations, \hat{N}_e observed in the Kerguelen population appeared similar to the \hat{N}_e provided by simulated populations with a random or a weak polygynous (i.e. 80% of males involved in reproduction) mating system.

Table 4 Variance in allelic change (F_k), N_e estimated with Waples' method and 95% CI, for genetic data collected in the Haute Island mouflon population and for simulated populations (F_k averaged over 1000 replicates \pm SD) using different combinations of mating system, generation times (T_g), lifespans, and juvenile mortality rates. Polygyny represents the proportion of males in the population that reproduce during a mating season

	Mating system	Life span	Juvenile mortality rate	F_k	N_e		
					$T_g = 2$ years	$T_g = 3$ years	$T_g = 4$ years
Observed	—	—	—	0.0332	208 (110–427)	138 (74–285)	104 (55–213)
Simulated	Random mating	3	0.25	0.0336 ± 0.0120	194 (104–364)		
		4	0.35	0.0315 ± 0.0083	218 (114–431)	146 (73–289)	
		5	0.45	0.0308 ± 0.0108	227 (118–454)	151 (69–242)	113 (52–182)
	Polygyny 0.8	3	0.25	0.0362 ± 0.0140	171 (94–306)		
		4	0.35	0.0333 ± 0.0091	197 (106–374)	131 (70–249)	
		5	0.45	0.0323 ± 0.0151	208 (110–400)	139 (73–269)	104 (55–201)
	Polygyny 0.5	3	0.25	0.0395 ± 0.0234	146 (83–252)		
		4	0.35	0.0349 ± 0.0138	182 (99–334)	121 (66–222)	
		5	0.45	0.0331 ± 0.0175	199 (106–377)	132 (71–251)	100 (53–189)
	Polygyny 0.2	3	0.25	0.0485 ± 0.0255	110 (64–176)		
		4	0.35	0.0419 ± 0.0146	136 (77–230)	90 (52–153)	
		5	0.45	0.0385 ± 0.0163	155 (87–271)	103 (58–180)	77 (43–135)

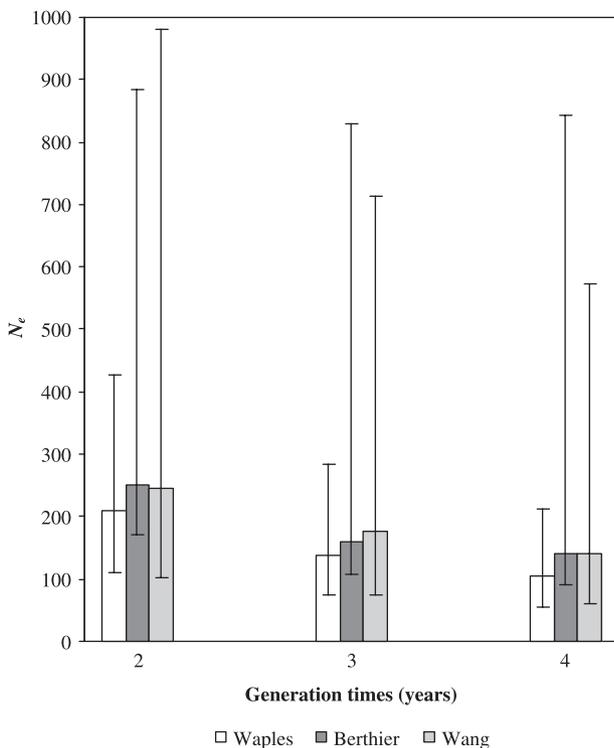


Fig. 2 Effective population size and 95% CI measured for the Haute Island population using Waples' (1989), Berthier *et al.*'s (2002) and Wang's (2001) methods for three generation times (2, 3 and 4 years).

Discussion

The observed \hat{N}_e (i.e. 104–250 individuals) measured with genetic methods were about two to three times higher than

the expected N_e derived from demographic methods (i.e. 58 individuals; Fig. 2 and Table 1). When the temporal method is used for species with overlapping generations, \hat{N}_e could be biased (Jorde & Ryman 1995). Recently, Waples & Yokota (2007) showed that the strength and direction of the bias is linked to the sampling method and to species life-history characteristics. Waples & Yokota (2007) showed that \hat{N}_e could be overestimated for large mammals, which are characterized by type I survivorship (i.e. high survival during adulthood followed by a rapid increase in mortality). However, they also showed that the bias is negligible if the two samples are separated by more than five generations as in our study. Furthermore, with its high overwinter mortality, the Kerguelen mouflon population more resembles type II or type III survivorship species which are generally associated with underestimated \hat{N}_e (Waples & Yokota 2007). For these different reasons, we believe that overlapping generations do not cause substantive bias in our \hat{N}_e estimations.

Genotyping errors can generate spurious results in population genetic analyses (i.e. Pompanon *et al.* 2005; Chapuis & Estoup 2007). However, in our case, it is unlikely that genotyping errors could have generated the difference between genetic and demographic estimates of N_e . We did not find evidence for departure from Hardy–Weinberg or large F_{IS} that could indicate the presence of null alleles. Furthermore, analyses that use allele frequencies, such as the estimation of effective population size, are less sensitive to genotyping errors than analyses that use genotype information to identify individuals (Pompanon *et al.* 2005).

Difference between genetic and demographic estimates could have been greater if we had been able to consider

variance in the lifetime reproductive success of individuals caused by promiscuous mating system in the demographic method (Nunney 1993). Genetic estimates gave a high \hat{N}_e/N ratio (0.38–0.77, Waples' methods, N is the total number of adults; equivalent to β_A) despite the cyclic demography and the strongly female-biased sex ratio. Our lowest estimate of 0.38 could be explained by the fact that we overestimated the generation time: large values of generation time leads to a decrease in the number of generations between samples. The drift measured is thus distributed on a shorter period, leading to a smaller \hat{N}_e . Using stable populations and the demographic method, Nunney (1991, 1993, 1995) predicted a theoretical value of N_e/N ratio close to 0.5. In contrast in his review on N_e/N , Frankham (1995) reported an average ratio of 0.11 in vertebrate species, including fluctuating populations. Our study shows that fluctuating population size reduces N_e estimate by only 10% ($\phi_A = 0.9$, Kalinowski & Waples 2002). This result may occur because minimum population sizes during the cyclic phase (see Fig. 1) were not small enough to create a real genetic bottleneck. Because the breeding season occurs before winter, many males may have transmitted their genes before the winter crash. Furthermore female survival and reproduction were not greatly affected by the crash (Boussès *et al.* 1994; Réale *et al.* 1999), and pregnant females could be considered as a reservoir of genetic diversity (Nunney 2002). These phenomena may have mitigated the loss of genetic diversity and therefore population dynamics could have a limited impact on the genetic estimates of \hat{N}_e .

The interaction between mating system and population dynamics could have complex effects on N_e : the demographic composition of a population may influence the variance in reproductive success between individuals (Pemberton *et al.* 1996; Coltman *et al.* 1999; Kokko & Rankin 2006). As with other sheep species, the mouflon is characterized by a promiscuous mating system (Bon *et al.* 1992) with high male–male aggression leading to a biased reproductive success among males. Mature rams are expected to have greater reproductive success than yearlings and young adult males (Bon *et al.* 1992; Coltman *et al.* 2002) even though young males can efficiently fertilize females when old males are rare in the population (Bon *et al.* 1992). Such a mating system with a high variance in male reproductive success is expected to decrease N_e (Hill 1972; Nunney 1993; Falconer & Mackay 1996). In the Kerguelen mouflon population, males suffered higher mortality than females during a winter crash (Boussès *et al.* 1994), probably as a result of their high reproductive effort before winter (see Geist 1971; Festa-Bianchet 1987; for example in big-horn sheep). This strong differential mortality between the sexes in the Kerguelen population created a strong bias in the secondary sex ratio. This type of pattern should increase the variance in reproductive success between

the sexes, further reducing N_e (Falconer & Mackay 1996). Following the 1988 crash, the mouflon population declined from 650 to 300 individuals (Boussès *et al.* 1994). Only three males (1.3% of the population) older than 2 years of age survived the crash (Boussès *et al.* 1994). Assuming that the surviving adult males were the only males that contributed to reproduction the following breeding season, we estimated the effect of a biased sex ratio on the annual breeding effective population size using the formulae $Nb = (4NmNf)/(Nm + Nf)$ (Falconer & Mackay 1996). Based on the distribution of individuals per age and sex classes in 1989 (Boussès *et al.* 1994), we estimated that the breeder's effective population size would have been reduced by about 91% compared to a population with a balanced sex ratio (Table 1). On a larger period, our study shows that the biased sex ratio should reduce the Kerguelen mouflon effective population size by 76% ($\alpha_A = 0.24$). Combined with population fluctuations observed in the mouflon population, biased sex ratio should reduced N_e by 79% ($\beta_A = 0.21$). However, after the inclusion of information on sex ratio and on population dynamics, N_e estimated with the demographic method still differed from the genetic estimates. The high genetic estimation of \hat{N}_e could therefore suggest that males less than 2 years old were involved in reproduction at low-density population. Observations during the rutting periods of 1991–1994 supported the hypothesis that 6-month-old lambs, yearlings and 2-year-old males were behaviourally involved in reproduction on Haute Island (D.R., personal observation). These findings are supported by behavioural and paternity studies on other ovids that showed that yearling and subadult rams contribute to reproduction as soon as the competition between males decreases (Stevenson & Bancroft 1995; Pemberton *et al.* 1996; Coltman *et al.* 1999). The mate guarding strategy primarily used by dominant male ovids, and the more efficient mate tactic in an aged-structured population (Hogg & Forbes 1997; Pelletier & Festa-Bianchet 2004), becomes inefficient with a highly female-biased sex ratio (Clutton-Brock 1988). Changes in demographic conditions favour mating tactics used by younger rams (i.e. 'courting', fighting to gain access to a female, or blocking, preventing a female to access a dominant male; Hogg & Forbes 1997) that are otherwise less efficient (Hogg & Forbes 1997; Coltman *et al.* 2002). Such an increase in the number of potential reproducing males after a crash would therefore limit the effects of the crash on N_e . When we included those yearlings in the number of potential reproducers, the demographic N_e went up to 147 individuals (Table 1). Including yearling and 2-year-old rams that survived the 1988 winter crash and could reproduce in 1989 in our estimate of N_e , the sex ratio was less biased. N_e in 1989 was thus reduced by only 58%, and on average by 49% on the whole study period ($\alpha_Y = 0.51$; Table 1). Adding young rams, to the estimation, did not change the effect of

population dynamics on the reduction in N_e ($\phi_Y = 0.87$ vs. $\phi_A = 0.90$). However, the cumulative effect of population dynamics and biased sex ratio reduced N_e by only 55% ($\beta_Y = 0.45$) compared to the 79% observed when adults alone were used. The similarity between these demographic estimates of N_e and the genetic \hat{N}_e suggests that yearlings were involved in the reproduction, and played a strong role in the dynamics of the genetic diversity in Kerguelen mouflon population.

To extend these results, we used a simulation approach that mimics the Kerguelen mouflon population. Our simulation results showed that the observed \hat{N}_e/N ratio at Kerguelen is similar to a \hat{N}_e/N ratio expected in a population under a random mating or with a slightly polygynous mating system (Table 4) (i.e. proportion of males that reproduce is > 0.5). About 50% of males may therefore be able to reproduce in the Kerguelen population. The large range of lifespans in the simulations allowed us to investigate the effect of overlapping generations on N_e . As predicted by Nunney (1993), the negative effect of high polygyny on N_e is dampened by a longer life span. In our simulated populations, where males breed at random, the increase in lifespan increases the chance that a male has to reproduce and therefore decreases the variance in lifetime reproductive success. Our results should, however, be considered with caution given the large confidence interval generally observed for \hat{N}_e estimations (for discussion see Waples 1989; Tallmon *et al.* 2004).

The combination of population dynamics and mating system can therefore have more complex effects on the N_e than each factor considered separately. Our results support Kokko & Rankin (2006) suggestion that population density plays an important role on mating strategies and their consequences on the dynamics of genetic diversity. Fluctuations in population structure and density can have a positive effect on N_e in a population characterized by a large variance in reproductive success between males, as exemplified in the Kerguelen mouflon population. Such a change in the variance in male reproductive success following demographic changes has been observed in similar conditions in the Soay sheep population (Coltman *et al.* 1999). Although they did not estimate N_e , Coltman *et al.* (1999) have suggested that under such conditions, the negative effect on N_e of polygynous mating system and population fluctuations could be minimized. Fluctuations in population density and structure may also affect N_e in other ways. For example, population density affects birth sex ratio and sex-dependent mortality early in life in ungulates species (Clutton-Brock *et al.* 1982; Kruuk *et al.* 1999) but its effects on N_e have not yet been examined.

Fluctuation in operational sex ratio should decrease N_e in monogamous species, because it should increase the variance in reproductive success among individuals in

the population (Nunney 1993). The impact on N_e in populations with a cyclical demography will differ between species as a result of different breeding phenologies (i.e. spring vs. fall mating periods) or life cycles (Nunney 2002; Waples 2002).

Island populations are usually genetically depleted compared to mainland populations (Frankham 1997a,b). A high N_e/N would therefore limit drift and potential for extinction (Newman & Pilson 1997). In ovids populations, dominant males generally monopolize the access to most oestrus females and sire a large proportion of the offspring produced each year (Coltman *et al.* 2002). Adult males generally prevent juvenile and young adult males from reproducing. Such a system is characterized by overt competition among males for access to oestrus females and sexual selection acts strongly on male fighting characteristics such as body or horn size (Coltman *et al.* 2002; Pelletier & Festa-Bianchet 2006). In the mouflon population, however, fluctuation in male age structure may have relaxed sexual selection pressures by allowing some males to contribute more than they could have done in a stable population. This decreased in selection pressures may therefore permit the maintenance of some deleterious alleles, which otherwise would have been eliminated (Whitlock 2000). From an evolutionary point of view, a small N_e illustrates increased risk of genetic drift and of inbreeding in a population (Newman & Pilson 1997), but is not always related to a lower evolutionary potential (McKay *et al.* 2001; Quader 2005). Ecological conditions favouring the reproduction of usual nonbreeders will therefore increase N_e , but will also relax selection and drive the population characteristics away from the optimum (Eisen *et al.* 1973), at least on the short term.

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