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Genetic neighbourhood and effective population size in the endangered European mink *Mustela lutreola*

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Abstract. Genetic neighbourhood and effective population size (N_e) are critical factors when determining the potential survival of threatened species. Carnivores have intrinsically small effective numbers, because, as top predators, they show low densities. The European mink, *Mustela lutreola*, is one of the most endangered carnivores in the world and has suffered continual decline and local extinctions. The genetic neighbourhood, area within which adults could randomly mate, averaged $N_a = 31.7$ km diameter, allowing that population size within the neighbourhood area only ranged from $N_b = 16.1$ to 22.8 animals. Although the population size was assessed in one of the main mink populations in the world, this neighbourhood size is far below the values regarded as critical in literature. However, in contrast with recent propositions, the ratio N_e/N only ranged between 0.09 and 0.19, estimates close to the average recognised by Frankham [(1995) Genetic Research 66: 95–107] for wildlife populations. In the context of the challenge to conserve this endangered carnivore, the studied neighbourhood provided crucial information suggesting both a low neighbourhood size and severe disturbance of breeding exchanges, emphasising the dramatically threatened status of the European mink.

Abbreviations: N_e – effective population size; N_a – genetic neighbourhood area; N_b – population size within the genetic neighbourhood area.

Introduction

For a declining population, genetic considerations for the conservation of endangered species focus on critical consequences of inbreeding depression leading to an extinction vortex (Lacy and Lindenmayer 1995). The decline in population results in an increase of inbreeding, which in turn may worsen the decline by depressing the fitness and restricting the evolutionary potential (Amos and Harwood 1998; Frankham 2003). The effective population size N_e basically influences conservation biology of wildlife populations because the effective size determines the loss in genetic variability due to drift at a rate of $1/2N_e$ per generation (Soulé 1976; Franklin 1980; Soulé 1987; Franklin and Frankham 1998). Furthermore, populations of small size are highly vulnerable to stochastic events (Soulé 1987; Caro and Laurenson 1994) and there has been a growing trend in conservation towards the use of both ecological and genetic approaches in order to understand how species are threatened.

Because N_e is typically smaller than the census wildlife population size, the assessment of genetic neighbourhood area is needed as a basic step procedure (Begon et al. 1980; Nunney 1993; Frankham 1995; Driscoll 1999). The genetic neighbourhood area N_a spans the area where animals could mate randomly and is related to the effective size of a population (Wright 1969). In a continuously distributed species, the range could exceed the neighbourhood area, but for endangered species with restricted distributions, the estimates of neighbourhood size give a decisive value because large zones are deserted by animals. Taking account of most of the essential factors determining population size, sex-ratio, reproductive success, adult lifetime, and generation overlap, Nunney and Elam (1994) proposed a 'demographic minimal model' to estimate the ratio of effective versus adult population size N_e/N. In the context of the challenge to conserve the endangered population, models estimating N_e provided crucial information regarding population viability (Morris et al. 1999) and are proved to have a strong heuristic value to define and prioritise appropriate conservation measures.

Carnivores basically exhibit small effective numbers because as top predators they are found in relatively low densities even in optimal conditions. Due to natural or human-induced processes, rarity constitutes one of the major patterns of the vulnerability of carnivores. The European mink, *Mustela lutreola*, is regarded as one of the most endangered mammals in the world. It has shown severe declines and local extinctions and the species is currently restricted to several vulnerable small populations in south-western France, northern Spain, eastern Romania, Russia and Belarus (Maran and Henttonen 1995). Although the regression of the European mink's eastern population is mainly attributed to intra guild competition (Maran et al. 1998), the decline in the mink's western population, as revealed by trapping and examination of skulls, was proved to be chiefly dependent on persecution and habitat alteration (Lodé et al. 2001). The endemic European mink western population had a recent history of population bottleneck, declining to half its previous range in less than 20 years between 1976 and 1999 (Lodé 1999; Lodé et al. 2001). The vulnerability of freshwater ecosystems to anthropic pressures caused more and more frequent decline, and fresh water predators were proved to be vulnerable to deteriorating watercourses. Moreover, mink exploited home-ranges along water course banks or marshes using from 2 to 6 km of riparian woods (Garin et al. 2002). It could be suspected that movements were favoured along watercourses or ditches leading to a mainly linear breeding dispersal in which subpopulation exchanges were facilitated between contiguous ranges. Following the stepping-stone model (Gadgil 1971), it could be predicted that such one-dimensional breeding dispersal should worsen the genetic depletion of such a critically endangered carnivore. Faced with such a serious decline, population genetics could provide critical guidance for future conservation planning (Ellstrand and Elam 1993; O'Brien 1994; Frankham et al. 2002).

This paper aims to investigate neighbourhood area and effective population size of endangered European mink, *M. lutreola*, two crucial pieces of information required to develop recovery strategies. In this study, we examined (1) the genetic neighbourhood area N_a and (2) the effective population sizes in order to explore how the decline of such a vulnerable species affected the ability to retain genetic diversity in a biological conservation perspective. The estimates of neighbourhood area provide an important guide for defining future protected areas devoted to mink conservation.

Methods

Neighbourhood area and population size

The genetic neighbourhood is the area within which adults could randomly mate, and the neighbourhood size is the number of adult animals within the neighbourhood area. From Wright (1969), genetic neighbourhood N_a is the spatial area defined as $2R = 2\sqrt{4/3 * S^2 * T}$, where S^2 is the variance in breeding displacement and T the adult lifetime period over which an animal may have offspring. Breeding displacements and variance were estimated from seven radiotracked mink surveyed by Garin et al. (2002). Because European mink did not use only river banks, but were found to exploit woods as well (Zabala et al. 2003), they did not exhibit a strict linear home-range (Zabala and Zuberogoitia 2003; Zabala et al. 2003). Therefore the use of models based on radial dispersion is required. The neighbourhood size was assessed by determining the number of adult mink N_b that fell within the area defined by $2R$. The number of adult mink within the neighbourhood area was estimated through a capture–recapture design carried out on the Seugne river and adjacent watercourses (Haute-Saintonge) between 1999 and 2002. A total of 70 wire-mesh traps were placed every 75–100 m in two lines along a 5–6 km stretch of rivers for 20 consecutive nights. Live-trapping sessions were carried out 17 times representing a total of 75 km of river bank-side. Field data included sex, age, weight, date and location of capture, and animals were released at the point of capture. Although the European mink was a rare critically endangered species, a total of 35 European mink *M. lutreola* were live-trapped on 58 occasions, representing the highest number of European mink ever captured in recent years. Therefore, the Haute-Saintonge may be one of the main mink subpopulations in the world.

Demographic model for N_e

Irrespective of neighbourhood size, the demographic minimal model (Nunney and Elam 1994) in population size is determined as

$$\frac{N_e}{N} = \frac{4r(1-r)T}{rA_f(1+I_{A_f}) + (1-r)A_m(1+I_{A_m}) + (1-r)I_{b_m} + rI_{b_f}}$$

where r is the male proportion of adult sex-ratio, T the generation time, A_f/A_m are, respectively, the female and male adult life span, I_{Af}/I_{Am} are female and male standardised variance in adult life span, I_{bf} and I_{bm} are female and male variance in

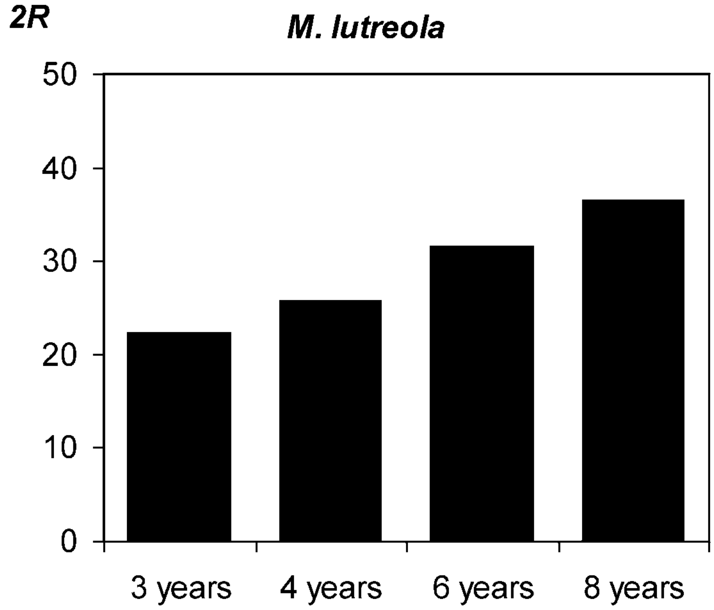

Figure 1. Estimation of the genetic neighbourhood N_a regarding different adult life times.

reproductive success per season. This estimation of N_e/N assumed that female fecundity and male mating success depended upon seasonal random factors, and supposed independent adult survival, that all adults attempt to breed and that juvenile survival to maturity did not differ from Poisson distribution law. Besides, based on the use of age or sex structure of the population, a stable structure through the years was assumed. Sex-ratio was determined from live-trapping sessions carried out from 1998 to 2001 and generation time was estimated as the average age of adults. European mink start breeding at one year old, the spring following their birth, thus the adult life time and mean reproductive success were derived from existing literature (Weber 1989; Maran 1990) and were assumed to be constant over the years. Several evaluations of N_e/N were performed incorporating distinct estimates of adult life span and variance in reproductive success for males and females.

Results

Neighbourhood and population size

A total of 35 adult mink were live-trapped in the study area reaching 0.44 mink/1 km of watercourse or 1 mink for 2.27 km of river bank side. Adult females predominated in populations with 59.1%. The sex-ratio of adults (number of males/number of females) only reached 0.69, emphasizing the dominance of mature females. From radio-tracking data, breeding displacement reached 9.67 km

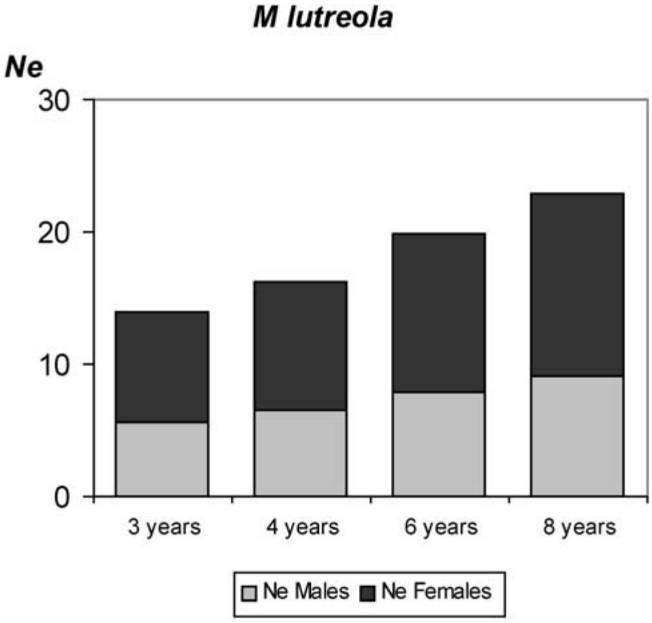

Figure 2. Estimate of population size within the neighbourhood area N_b regarding different adult life times and based on a sex-ratio = 0.69.

(range = 0.6–16.8, SD = 5.59) for a variance of 31.3 km. Based on mean variance, the genetic neighbourhood N_a was estimated to be $2R = 31.65$ km but could vary regarding different adult life times (range = 22.4–36.5, Figure 1).

The estimate of population size within the neighbourhood area ranged at a very low value from $N_b = 14.0$ to $N_b = 22.8$ animals (Figure 2). Anyway, the neighbourhood size showed a very low value, lower than 100 animals.

Ratio N_e/N

The proportion of males in the mink population was estimated as 0.409. Based on age structure of the population, estimates of adult life span were 7 years for females and 6 years for males. Based on 7 and 6 years of adult life span, the ratio of effective population size to adult population size only reached $N_e/N = 0.109$. Based on equal adult life span with, respectively, 6 years or 8 years, the ratio ranged from $N_e/N = 0.115$ to 0.089. The estimate only increased to $N_e/N = 0.158$ or $N_e/N = 0.197$, respectively, regarding the adult life span at 4 years and at 3 years.

Discussion

The salient issue for conservation of rare carnivores is the need for extensive natural areas supplying abundant resources, and in which a functional connectivity by breeding dispersal among subpopulations is allowed. Here, the small effective neighbourhood size and the ratio N_e/N emphasised that the population was divided into very small units unable to prevent inbreeding depression.

The European mink western population was not evenly distributed over its current range, but exhibited a pattern critically fragmented into some distant subpopulations (Lodé 2002). In endangered species, patterns of distribution were obviously dependent on habitat quality (Bright and Smithson 2001; Lodé 2002; Zabala et al. 2003). Because they are at the top of the trophic chains, freshwater predators may be especially vulnerable to deteriorating water quality and mink avoidance from altered watercourses resulted in this worsened subdivision of population (Lodé 2002). In the same way, otter (*Lutra lutra*) populations were significantly partitioned among sampling locations (Randi 2003). Nevertheless, mink are found to be highly mobile animals (Garin et al. 2002; Zabala and Zuberogoitia 2003) and restriction of breeding dispersal was not clearly evidenced. Where populations did not have a discrete distribution but showed a continuous or a semicontinuous distribution, the genetic neighbourhood provided crucial information because panmixy was not credible on the continuum. Based on adult dispersal, values for genetic neighbourhood N_a reached more than 30 km, a result apparently consistent with natural breeding exchanges. The neighbourhood area governs the relative influence of gene flow, but the neighbourhood area found in European mink comprised a spatial area including less than $N_b = 25$ adult animals. The home-range of mink resulted in a scattered distribution comprising a low number of breeding animals, one of the smallest reported for vertebrates. The size of neighbourhood area encompassed a very low number of mink restricted to the riverside, and the scattered distribution of animals could be regarded as a real threat for small isolated subpopulations. Thus, the low number of European mink within the neighbourhood area suggested that the conservation of the species may be affected by a favourite one-dimensional dispersal strategy, restricting breeding dispersal as in a stepping stone model.

Because some dominant individuals can control resource availability or breeding, N_e often showed a value lower than 5–10% of N (Nunney 1993; Frankham 1995). All considerations dealing with the conservation strategies suggest that an effective size of $N_e = 500$–1000 is required to retain evolutionary potential and to avoid inbreeding depletion (Franklin 1980; Lande and Barrowclough 1987; Frankham 1995; Franklin and Frankham 1998), and as large as $N_e = 1000$ individuals to avoid accumulation of deleterious mutations (Lande 1995; Lynch and Lande 1998). Thus, the neighbourhood size found in European mink was very low, below $N_e = 100$. It is uncertain that a long-term viable mink population can be maintained in such a restricted neighbourhood area. However, N_b is not a real measure of effective population size and only generated information about the spatial scale of potential breeding dispersal.

Numerous studies have focused on demographic parameters regarded as influencing N_e/N estimates (Franklin 1980; Waite and Parker 1996). Reviewing 14 studies, Nunney and Elam (1994) found that the ratio of N_e/N averaged 0.73 using their minimal method. Nunney (1993, 1995) emphasised that N_e/N should have a value of 0.5 and did not fall beyond 0.25. Frankham (1995), however, reported mean estimates of N_e/N of 0.11 from 192 studies on unmanaged wildlife species and concluded that fluctuations in population size are likely to decrease the ratio N_e/N in the long term. Changes in reproductive success could influence estimates of the ratio, a factor expected to be especially strong in highly fecund species (Nunney 1996; Bekkevold et al. 2002). However, our results showed that sex-ratio or breeding success did not significantly affect the ratio in the mink. In marten, Bright and Smithson (2001) argued that stochastic factors on mortality and sex-ratios are most likely to establish population survival, while factors affecting birth rates will probably most influence spread.

Frankham (1995) found that most main life-history characteristics have little influence on N_e/N and identified fluctuation in population size as the most determinant variable. Although the conservation challenge should be to increase the ratio by a manipulation of parameters, these effects are suspected to have both little influence and to be very difficult to produce. Because most mink breed in their first year, it seems technically impractical to improve their generation time. The sex-ratio is consistent with a polygynous mating system such as found in the genetically related species, the European polecat (Lodé 2001). Furthermore, given the degree of variation in demographic parameters, there are reasons to suggest that the ratio N_e/N may be overestimated. Firstly, variations in reproductive success of animals over the years may increase the variance and thus lessen the ratio. Secondly, the Allee effect hypothesis (Allee et al. 1950) predicted that poor habitats may result in extensive scattered home-ranges and that low densities prevent most females from finding mates. Because of poor habitats ensued from bad watercourse quality, mink may have widely spaced ranges. This suggests that many adults may fail to breed, supporting the Allee effect hypothesis, while all adults are expected to mate in the model. Thirdly, the average adult life span is estimated to reach from 3 to 8 years (a canonical age for most mustelids; Weber 1989) but the ratio always remains very low, below the ratio of 0.25. In addition, while dispersal has been shown to enhance the survival of small populations through a ‘rescue effect’, the intolerance of individualistic mustelids may affect their dispersal (Lodé et al. 2003). The view for European mink conservation needs to take account of the low N_e/N and an urgent plan for a real conservation is critically required.

Carnivores such as otters or mink require specific habitat conditions but, as top predators, they are always found in low densities, even in optimal habitats, and intrinsically exhibit a strong vulnerability to demographic depletion. The European mink genetic neighbourhood may be affected by the one-dimensional dispersal. Long-distance dispersal events may significantly reduce inbreeding depression and are biologically very important for small population survival. However, long-distance dispersal was found in a very low frequency among animals and was drastically dependent upon functional habitat conservation. Although low values for genetic neighbourhood may mostly result from mink ecology, undoubtedly, long-term recovery of the critically endangered *M. lutreola* western population depends upon the preservation of such extensive breeding dispersal and urgently required that large protected areas were really delineated.

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