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Population genetics

Increase of heterozygosity in a growing population of lesser kestrels

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The lesser kestrel (*Falco naumanni*) suffered a sharp population decline over much of its European distribution range in the middle of the twentieth century. Still declining in some areas, the species has recently experienced a notable population recovery in certain regions. We examined the genetic diversity variation in a growing population of lesser kestrels from Central Spain over a 6-year period (2000–2005). The population studied showed a rapid demographic expansion, increasing in the number of both breeding pairs and colonies. Annual average heterozygosity and allelic diversity increased and genetic similarity between potential mates decreased over the study period. Several immigrants regularly arrived in the study area and introduced new alleles into the local population, pointing to immigration as the main cause contributing to the observed genetic recovery.

Keywords: bottleneck; conservation genetics; genetic diversity; microsatellites

1. INTRODUCTION

The genetic consequences of population size are a central topic in conservation biology (Frankham 1996; Saccheri *et al.* 1998). Differences in genetic variability among stable populations varying in size have been documented for several species (Frankham 1996). However, temporal patterns of genetic diversity within expanding populations have hitherto been rarely reported, probably due to the difficulty of long-term monitoring of populations across years (Hansson *et al.* 2000; Vila *et al.* 2003; Kaeuffer *et al.* 2007). Translocation experiments suggest that the contribution of only a small number of 'immigrants' can induce important changes in local genetic diversity (Saccheri & Brakefield 2002) and rescue genetically depauperate populations (Westemeier *et al.* 1998; Madsen *et al.* 1999). Thus, data on recovery of natural genetic diversity are of great importance for predicting population growth and viability once negative factors inducing population decline have disappeared (Saccheri *et al.* 1998; Keller *et al.* 2001; Vila *et al.* 2003).

The lesser kestrel (*Falco naumanni*) is a colonial and migratory falcon. This species was once one of the most abundant birds of prey in Europe, but it suffered a sharp population decline in its western Palearctic breeding range in the second half of the twentieth century that led to a complete extinction in several countries and strong declines in others (Biber 1990). The Spanish population also experienced a severe population crash, as it dropped from an estimated 100 000 pairs in the 1960s to 4000–5000 breeding pairs in late 1980s (González & Merino 1990). Still declining in several countries, the lesser kestrel has experienced recent demographic expansions in other areas such as Mediterranean Spain and Southern France (e.g. Prugnolle *et al.* 2003; present study). Here, we examined the change in genetic diversity over a 6-year study period in a growing and expanding population of lesser kestrels from Central Spain.

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2. MATERIAL AND METHODS

(a) *Population monitoring*

Since 1993, we have monitored an area covering approximately 1000 km^2 in La Mancha, Central Spain (39°20′ N, 3°15′ W), which is inhabited by a lesser kestrel population. Here, we focus on a 6-year period (2000–2005) in which we carried out a more intensive monitoring of the population and blood samples for DNA analyses were collected. Monitoring included the capture and banding of breeding adults and intensive ringing of nestlings in the colonies (Ortego *et al.* 2007*a*,*b*). In the study area, lesser kestrels form colonies of between 1 and 60 pairs located in abandoned farmhouses where they nest under tiled roofs or inside holes in walls. Every year, we estimated population size as the sum of the pairs of the colonies located in the study area (Aparicio *et al.* 2007). Further, we determined colonization events by prospecting the study area across years and recording new settlements in previously unoccupied buildings.

(b) *Genetic analyses*

We genotyped 937 individuals from 2000 to 2005 at the microsatellite loci Fp5, Fp13, Fp31, Fp46-1, Fp79-4, Fp86-2, Fp89 (Nesje *et al.* 2000), Fu1, Fu2 (J. H. Wetton 2000, unpublished data), Fn1-11 and Fn2-14 (Ortego *et al.* in press). Of these, 432 were nestlings and 505 were adult individuals breeding in the study area. Among these breeding individuals, 64 were first year un-ringed immigrants. Briefly, we used PCR to amplify dye-labelled microsatellites and quantified fragment size using an ABI 310 genetic analyser and the analysis program GeneScan v. 3.7 (Ortego *et al.* 2007*a*). We used two metrics to estimate heterozygosity: (i) uncorrected heterozygosity (H_O), calculated as the proportion of loci at which an individual is heterozygous and (ii) homozygosity by loci (HL), a new measure that weights the contribution of each locus to the homozygosity value depending on their allelic variability (Aparicio *et al.* 2006). HL is calculated as follows: $HL = (\sum E_h)/(\sum E_h + \sum E_j)$, where E_h and E_j are the expected heterozygosities of the loci that an individual bears in homozygosis (h) and in heterozygosis (j), respectively. H_O and HL were calculated using Cernicalin, an excel spreadsheet available on request.

(c) *Statistical analyses*

We tested for directional changes over time in population size (measured as the number of breeding pairs), annual average H_O and HL and total number of alleles using linear regression analyses. The total number of alleles summed over loci was standardized for sample size using a bootstrapping procedure (see electronic supplementary material for details). We performed analyses of heterozygosity taking into account that data provided by siblings are non-independent between them. Thus to avoid pseudoreplication, we dealt with the means of heterozygosity for each brood and then calculated average heterozygosity for each studied cohort (e.g. Kaeuffer *et al.* 2007). The precision of annual average heterozygosity could be different because sample sizes used for their estimation varied between cohorts. Hence, we used sample size to give observations different weights in a weighted least-squares analysis (e.g. Kaeuffer *et al.* 2007). Finally, we tested variation of within-pair genetic similarity over time for a possible directional change. We calculated average similarity (using the similarity index; Li *et al.* 1993) between all pairwise combinations of breeding males and females within colonies (e.g. Hansson *et al.* 2000). Colony identity was included as random effect in this last analysis to control the possible non-independence of genetic similarity within colonies. All tests are two tailed and were performed in SPSS v. 7.5.

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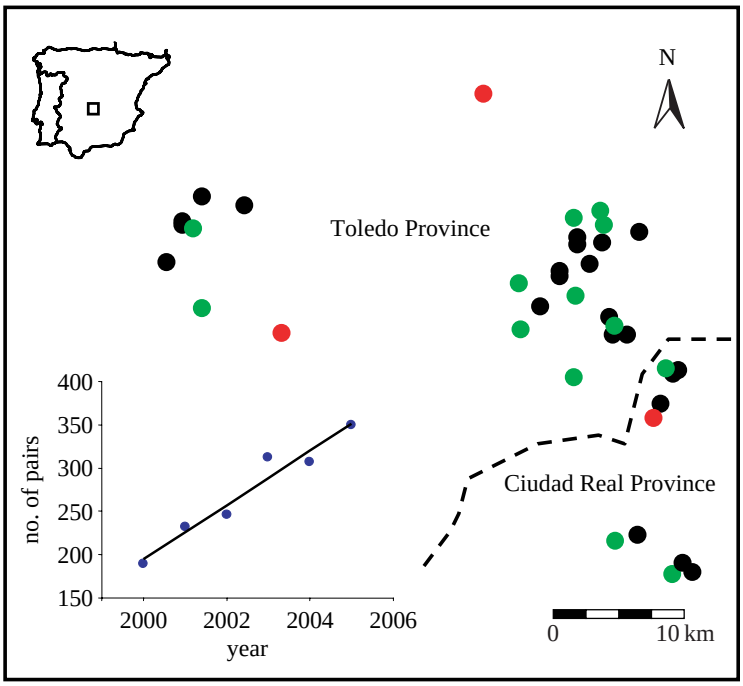

Figure 1. Map of the study area showing the spatial distribution of lesser kestrel colonies in 2000–2005. Colonies present in the study area since 2000 (black dots), founded after 2000 (green dots) and disappeared since 2000 (red dots) are indicated. Graph illustrates the increasing number of breeding pairs from 2000 to 2005.

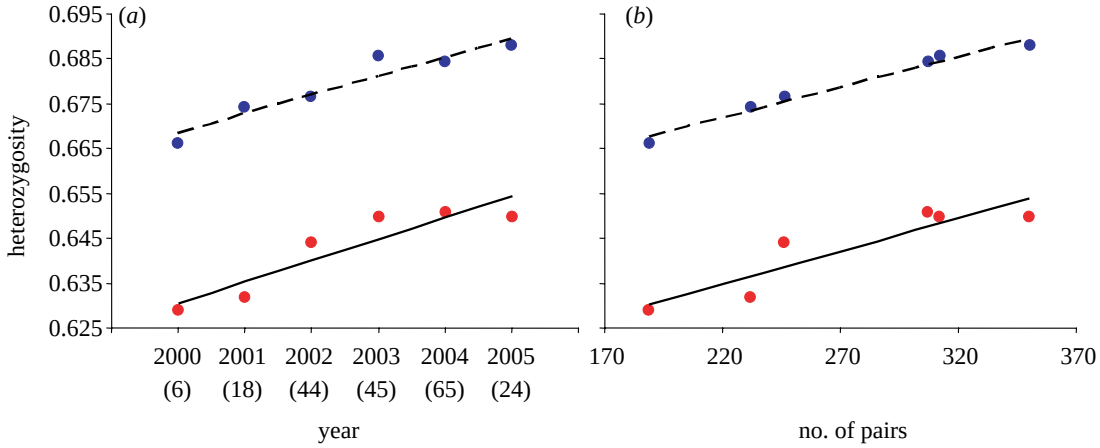

Figure 2. Annual average heterozygosity (H_O: red points, solid line; 1−HL: blue points, dashed line) in relation to: (*a*) year of study; (*b*) number of breeding pairs in the study lesser kestrel population. Sample size (i.e. the number of genotyped broods) used for calculating annual average heterozygosity is shown in parentheses for each year.

3. RESULTS

The number of breeding pairs in the study area has increased significantly since 1993 ($r=0.922$, $n=10$, $p<0.001$; years with available data: 1993, 1997, 1999–2006) and a similar trend occurred within the 6-year study period (2000–2005) with available genotypic information ($r=0.974$, $n=6$, $p=0.001$; figure 1). This means a 42% increase of breeding pairs since 1993 and a 54% from 2000 to 2005, with an average increase of 11% pairs per year in this last period. Furthermore, 13 new colonies have been founded in the study area from 2000 to 2005 whereas only three have disappeared in this period (figure 1). In all cases, colonies disappeared when buildings or roofs collapsed owing to human intervention (Calabuig *et al.* 2007). The values of annual average H_O and HL increased, respectively, by 6.50 and 6.95% over the study period (H_O: $r=0.852$, $n=6$, $p=0.031$; HL: $r=-0.907$, $n=6$, $p=0.012$; figure 2*a*). Allelic diversity increased on average at all 11 loci through the study period (student's *t*-test: $t=2.43$, $n=11$ loci, $p=0.036$). However, although total number of alleles summed over all loci tended to increase with years, the association was not significant ($r=0.654$, $n=6$, $p=0.159$). Annual average heterozygosity was positively associated with the number of breeding pairs in the study area in 2000–2005 (H_O: $r=0.842$, $n=6$, $p=0.036$; HL: $r=-0.922$, $n=6$, $p<0.001$; figure 2*b*), but we found no significant effect of population size on total number of alleles ($r=0.564$, $n=6$, $p=0.244$). After controlling the effects of colony identity, average similarity between all pairwise combinations of breeding males and females within colonies declined over time ($F_{1,38}=5.36$; $p=0.026$). Using the genotypes of individuals

present in the study area from 2000 to 2002 as a reference population ($n=233$ individuals), we found that several immigrants continuously carried novel alleles into the population (year, number of immigrants captured, number of novel alleles: 2003, 16 individuals, 3 alleles; 2004, 32 individuals, 12 alleles; 2005, 16 individuals, 4 alleles) whereas 13 alleles presented in 2002 were absent in the last 3 years. Hence, there was a net gain of six alleles over the study period.

4. DISCUSSION

We found a concurrent increase in population size and annual average heterozygosity over the years 2000–2005 in a lesser kestrel population from Central Spain. The study population is not likely to have escaped from the widespread population decline registered for this species in the Iberian Peninsula. The growing trend in number of breeding pairs and colonies represents a population recovery from this crash, once some of the factors promoting population regression (e.g. organochlorine pesticides, direct human persecution, etc.) diminished.

Increasing population size could have contributed to an augmentation of mean population heterozygosity by diminishing the chance of crosses between relatives (Vila *et al.* 2003). The increased genetic diversity and reduced similarity between potential mates over time may probably be associated with the observed arrival of immigrants regularly introducing novel alleles into the local population (Hansson *et al.* 2000; Keller *et al.* 2001). The arrival of immigrants may have been facilitated by the fact that the study population is located in the core of the species distribution in the Iberian Peninsula which may have favoured immigration processes compared with populations located in marginal environments or near the edges of the species geographical distribution. Further, the relatively short generation time of lesser kestrels (modal lifespan is 4 years) could have favoured a rapid settlement of immigrants as consequence of a more frequent turnover of individuals in the colonies in comparison with long-lived territorial species (Hailer *et al.* 2006). Apart from the possible effects of immigration, other factors could have contributed to the observed increase in genetic diversity across years. First, different components of fitness are affected by genetic diversity in the study population (Ortego *et al.* 2007*a*,*b*), suggesting that natural selection against more homozygous individuals could have also contributed to some extend to increase mean heterozygosity over years (Kaeuffer *et al.* 2007). Second, offspring born from crosses between immigrants and natives would benefit from hybrid vigour which may have favoured a rapid spread of immigrant genomes and accelerated the increase in genetic diversity over time (Saccheri & Brakefield 2002).

We manipulated and banded lesser kestrels under license from the Spanish Institutional authorities.

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