Strong philopatry derived from capture–recapture records does not lead to fine-scale genetic differentiation in lesser kestrels

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Summary

1. The integration of capture–recapture and molecular approaches can improve our understanding of the consequences of habitat fragmentation on population connectivity. Here we employed microsatellites to test dispersal hypotheses derived from intense and long-term ringing programmes of the lesser kestrel *Falco naumanni* in Western Europe.

2. Re-encounters of 1308 marked individuals in Spain have revealed that most first-time breeders settled within 10 km from their natal colony, with a negative association between dispersal and geographical distance. Although these findings would predict fine-scale spatial patterns of genetic differentiation, the genetic impact of rarely reported events concerning long-distance effective dispersal (> 100 km) is unknown.

3. First, we investigated a spatially structured and geographically isolated population located in north-eastern Spain, where capture–recapture records and genetic data could be appropriately compared over similar spatial and temporal scales. Spatial autocorrelation analyses (N = 174 nestlings from different broods) did not reveal either significant differences in average relatedness at any distance class nor decreased relatedness as a function of distance. At a broader spatial scale, Bayesian analysis of population structure (N = 432 nestlings) indicated panmixia across Western Europe. However, F_{ST} comparisons between four geographically distinct populations indicated low but significant genetic differentiation.

4. Our genetic data would therefore challenge traditional assumptions associating philopatry with the emergence of fine-scale genetic structuring. This could be because even low levels of gene flow are enough to preclude the development of local genetic structure. Nevertheless, the analysis of a geographically isolated and small population from Southern France exemplifies a situation in which restricted dispersal has translated into weak but consistently significant genetic differentiation.

5. Relevant to conservation genetics and evolutionary biology, our results may lessen the genetic concerns derived from population fragmentation at relatively small geographical scales in species with apparently limited dispersal abilities, but raises concerns about increased genetic divergence in small and isolated demes.

Key-words: genetic structure, gene flow, capture–recapture, conservation genetics, genetic diversity

Introduction

Dispersal of individuals can influence the genetic structure, demography and long-term persistence of populations (Young & Clarke 2000; Clobert *et al*. 2001). The use of molecular approaches and the development of powerful statistical methods have revolutionized the study of dispersal by providing an alternative that addresses many methodological limitations of traditionally laborious capture–recapture studies (see Koenig, Van Vuren & Hooge 1996). For example, long-distance dispersal events are difficult to document with capture–recapture techniques, but genetic methods have proved efficient in this task (e.g. Paetkau *et al*. 1995; Vilà *et al*. 2003). Genetic approaches, however, may fail to reflect current gene flow as well as non-effective dispersal movements of crucial importance, for instance, in spreading diseases. In addition, indirect measures of dispersal are often difficult to interpret because different population-level processes may result in similar genetic patterns (e.g. Bossart & Prowell 1998; Whitlock & McCauley 1999, but see Manel, Gaggioti & Waples 2005). Thus, it is not surprising

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that ecological and genetic methods have sometimes yielded conflicting results (e.g. Van Bekkum *et al*. 2006; Senar *et al*. 2006). Combining capture–recapture and genetic inferences in the same study models can help explain these discrepancies, and such integration may be critical in dealing with basic ecological and evolutionary questions (e.g. Gompper, Gittleman & Wayne 1998; Peacock & Ray 2001; Berry, Tocher & Sarre 2004; Hansson, Bensch & Hasselquist 2004; Double *et al*. 2005; Temple, Hoffman & Amos 2006).

The majority of species suffers from the effects of habitat loss and reduction due to human activity. The demographic and genetic consequences of habitat fragmentation depend on the interaction between the dispersal ability of the species and the number, size and spatial distribution of local populations, as well as on time since fragmentation (Young & Clarke 2000; Frankham, Ballou & Briscoe 2002). While restricted gene flow typically leads to genetic differentiation among fragments, a spatially structured population will behave similarly to a single large panmictic population if sufficient dispersal and associated gene flow rates are occurring (see Mills & Allendorf 1996; Vucetich & Waite 2000). In this respect, natal and breeding philopatry, i.e. the tendency of individuals to breed close to their birthplace or their previous breeding territory, are relevant life-history traits expected to generate population differentiation (Greenwood 1980; Greenwood & Harvey 1982; Sugg *et al*. 1996).

Intense and long-term monitoring of marked individuals in the globally vulnerable (BirdLife International 2007) and facultatively colonial lesser kestrel *Falco naumanni* in Spain revealed that the frequency distribution of movements is distance-dependent, with a majority of birds breeding for the first time within 10 km from their natal colony. This pattern of natal dispersal was independently documented for both a population located in the Guadalquivir Valley (69%, $N = 321$ individuals; Negro, Hiraldo & Donázar 1997, see Fig. 1), and a population located in the Ebro Valley (66%, $N = 751$, Serrano *et al*. 2003, Fig. 1). Apparently, only a few birds (~1%) settled at distances greater than 100 km from their natal colony (1% and 0·6% in the Guadalquivir and Ebro Valleys respectively, Negro *et al*. 1997; Serrano *et al*. 2003).

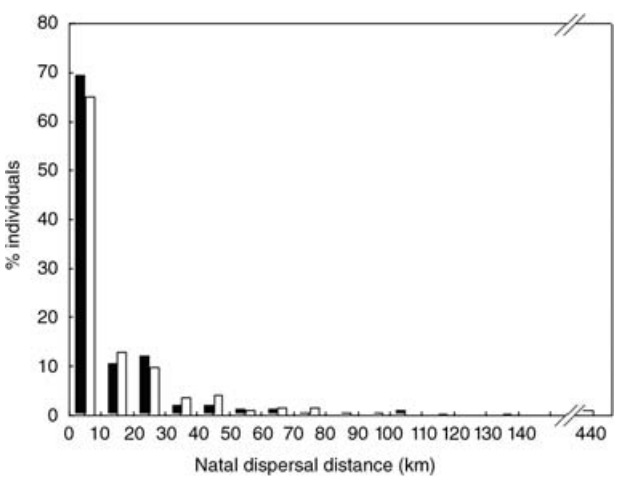

Fig. 1. Frequency distribution of natal dispersal distances of lesser kestrels in the Guadalquivir Valley (SW Spain, $N = 321$ individuals, black bars; Negro *et al*. 1997) and in the Ebro Valley (NE Spain, $N = 961$, white bars; Serrano *et al*. 2003).

Contrary to most avian studies, natal dispersal was shown to be not, or only slightly, sex biased (Negro *et al*. 1997; Serrano *et al*. 2003), and the potential differences between sexes have been preferentially attributed to their different roles in nest acquisition and defence, rather than to the development of effective mechanisms of inbreeding avoidance (see more details in Negro *et al*. 1997; Serrano *et al*. 2003). Natal dispersal has also been shown to be linked to density-dependent factors (Negro *et al*. 1997; Serrano *et al*. 2003, 2004), with settlement decisions of first-breeding birds being constrained in the largest colonies by agonistic interactions with previous residents (Serrano & Tella 2007). After monitoring 486 consecutive breeding attempts in the Ebro Valley, high philopatry was also documented in adult birds, with most kestrels remaining faithful to the colony in which they bred the year before (71·6%, Serrano *et al*. 2001). Adult females seemed to disperse more often than males (34% vs. 19%), and both sexes apparently dispersed less with age and experience (Serrano *et al*. 2001). Moreover, most of the dispersing adults settled within 2 km of their previous colony, and just 6% of them dispersed more than 10 km from their previous colony (Serrano *et al*. 2001).

Although strong philopatry and restricted dispersal over short distances are expected to generate fine-scale, nonrandom spatial patterns of genetic differentiation (Greenwood 1980; Greenwood & Harvey 1982; Sugg *et al*. 1996), the extent and genetic impact of long-distance dispersal in our study model is poorly understood. Thus, the key question that this article will address is whether local estimates of dispersal relying on capture–recapture data are good predictors of spatial patterns of genetic differentiation at two spatial scales using microsatellite markers. In order to address this question, we first employed an individual-based spatial autocorrelation analysis to investigate fine-scale genetic structuring in the demographically monitored and spatially structured population located in the Ebro Valley (see e.g. Serrano & Tella 2003; Serrano *et al*. 2005). This population is also geographically isolated (see Fig. 2), and both immigration from and emigration to other populations have been rarely documented by direct observations. Based on this evidence of limited gene flow, we then employed population-based analyses to test for genetically distinct clusters at a wider geographical scale covering the entire distribution range of the species in Western Europe.

Materials and methods

STUDY SPECIES AND POPULATIONS

Lesser kestrels are small migratory falcons that breed in Eurasia and winter in Africa (Cramp & Simmons 1980). Mostly monogamous, first breeding of lesser kestrels takes place at 1–2 years of age (Serrano *et al*. 2003). Levels of extra-pair paternity are in the lower range typical of raptors (7·25%, Alcaide *et al*. 2005; see also Korpimäki

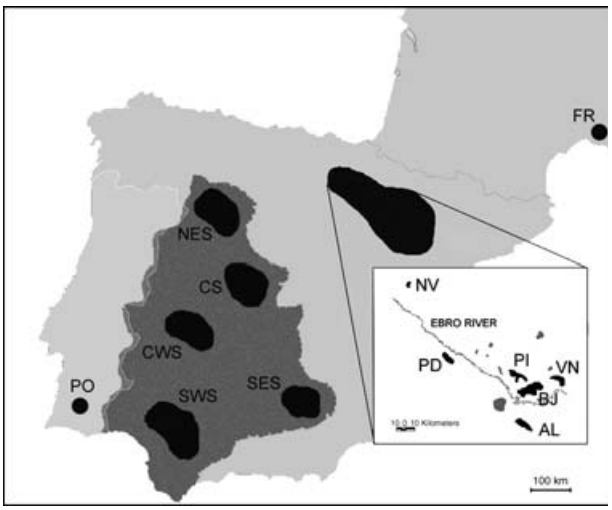

Fig. 2. Breeding distribution of the lesser kestrel in Western Europe. Dark grey areas represent the distributional range of the species. Black areas include sampled locations. The Ebro Valley population (north-eastern Spain) has been enlarged. Reintroduced populations are not indicated in this map. See Table 1 for location codes.

Table 1. Summary of the origin of lesser kestrel nestlings sampled for genetic analyses in Western Europe. Location code includes within brackets whether the sampling colonies were at the Ebro valley (EB), the Spanish core area (CA), the French (FR) or the Portuguese population (PO). See Fig. 1 for geographical locations

Location	Location code	Number of sampled colonies	Number of sampled nestlings
Navarra	NV (EB)	2	21
Pedrola	PD (EB)	1	16
Pina	PI (EB)	6	25
Bujaraloz	BJ (EB)	6	48
Ventas	VN (EB)	7	20
Alcañiz	AL (EB)	13	44
North-western Spain	NWS (CA)	11	34
Central Spain	CS (CA)	6	27
Central-western Spain	CWS (CA)	14	53
South-western Spain	SWS (CA)	14	64
South-eastern Spain	SES (CA)	11	29
Portugal	PO (PO)	2	25
France	FR (FR)	1	26
Total		95	432

et al. 1996; Arsenault, Stacey & Hoelzer 2002). This fact increases the probability that adult males will raise their own offspring and reduces the probability of sampling half-sibs from different broods. Average life span for lesser kestrels is 3–4 years, but some individuals are known to have lived more than 10 years (see Negro 1997 for more information on the species).

Our first spatial scale of analysis covers 10 000 km^2 in an 8-year (1993–2000) demographically monitored population located in the Ebro Valley, north-eastern Spain (Fig. 2). This region contains a recently founded (*c*. 1960), fast-growing and spatially structured population of lesser kestrels (see Serrano & Tella 2007 for details). Lesser kestrels breed there exclusively in farmhouses containing a variable number of pairs (1–43), and these colonies aggregate into different subpopulations (see Serrano & Tella 2003, Fig. 2). Our second spatial scale covers the species' distribution range in Western Europe, where four distinct populations can be defined on the basis of geographical criteria: Ebro Valley, Spanish core area, Portugal, and France (Figure 2). In the main Spanish core area, samples were obtained from different localities comprising the distribution borders [North-eastern Spain (NES), Central Spain (CS), South-eastern Spain (SES), South-western Spain (SWS) and one central region, Central-western Spain (CWS); see Table 1, Fig. 2]. Despite the population decline experienced by the species during the second half of the 20th century (Biber 1990), this population remains the largest population in Western Europe (about 12 000–19 000 breeding pairs, BirdLife International 2007). The current Portuguese population, estimated to be less than 300 breeding pairs, is concentrated in the south of the country and constitutes the south-western border of the species' distribution in Eurasia (Fig. 2). This population is currently recovering from a population bottleneck (see Alcázar & Henriques 2006 for details). Finally, the most geographically isolated breeding population of our study is located in Southern France (Fig. 2). This population was near extinction at the end of the 1970s (Cheylan 1991) but it has undergone a geographical and demographic expansion during the last two decades, reaching around 180 breeding pairs in 2007 (see Biber 1990; Pilard & Brunn 1998, http://crecerellette.lpo.fr/population/population.html).

GENETIC SAMPLING AND DNA EXTRACTION

During the 2002 and 2003 breeding seasons, blood or feathers were taken from 432 nestlings, each individual belonging to a different brood and presumably unrelated to all other sampled individuals. All individuals sampled from the same colonies belonged to the same cohort. Thus, we sampled 432 nests from 95 breeding colonies located in Spain, France and Portugal (see Table 1). Blood samples were preserved in absolute ethanol and feathers pulled from the nestlings' back were stored in paper. High concentrations of DNA can be obtained from growing feathers, given that tips are irrigated with a large number of blood vessels. Both types of samples were placed at 4 °C until processing. The extraction protocol follows that described by Gemmell & Akiyama (1996). Blood and feather tips were digested by incubation with proteinase K for at least 3 h. DNA purification was carried out using 5 M LiCl, organic extraction with chloroform-isoamyl alcohol (24:1) and DNA precipitation with absolute ethanol. Pellets obtained were dried and washed twice with 70% ethanol and later stored at –20 °C in 0·1 mL of TE buffer.

MICROSATELLITE GENOTYPING

We amplified nine microsatellite markers originally isolated in the peregrine falcon *Falco peregrinus*. Loci Fp5, Fp13, Fp31, Fp46-1, Fp79-4, Fp89 and Fp107, developed by Nesje and co-workers (2000), have been shown to be suitable for genetic studies in other *Falco* species (e.g. Groombridge *et al*. 2000). We also designed two sets of primers flanking two microsatellite sequences available in GenBank (AF448412 and AF448411, respectively). Locus Cl347 was amplified using primers Cl347Fw: TGTGTGTGTAAGGTTGCCAAA and Cl347Rv: CGTTCTCAACATGCCAGTTT. Locus Cl58 was amplified using primers Cl58Fw: TGTGTCTCAGTGGGGAAAAA and Cl58Rv: TGCTTTGGTGCTGAAGAAAC. For each locus, the polymerase chain reaction (PCR) was carried out in a PTC-100 Programmable Thermal Controller (MJ Research Inc., Waltham, MA, USA) using the following PCR profile: 35 cycles of 40 s at 94 °C, 40 s at 55 °C, 40 s at 72 °C and finally, 4 min at 72 °C. Each 11-μL reaction contained 0·2 U of *Taq* polymerase (Bioline), 1× manufacturer-supplied

PCR buffer, 1·5 mm $MgCl_2$, 0·02% gelatine, 0·12 mm of each dNTP, 5 pmol of each primer and approximately 10 ng of genomic DNA. F-Primers were 5′ end labelled with HEX, TET or 6-FAM. Amplified fragments were resolved on an ABI PRISM 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

GENETIC ANALYSES

Polymorphism statistics (i.e. number of alleles and observed heterozygosities) at each microsatellite marker were calculated with the software genetix 4·04 (Belkhir *et al*. 1996–2002). Conformity to Hardy–Weinberg expectations was analysed through genepop (Raymond & Rousset 1995), using a single locus and a global multi-locus test for heterozygosity deficit or excess by the Markov chain method (Raymond & Rousset 1995). Linkage disequilibrium was also tested with genepop.

Fine-scale genetic structuring in the Ebro Valley population (N = 174 individuals, Table 1) was investigated with a spatial autocorrelation analysis that was performed using the macro of the genalex package version 6 (Peakall & Smouse 2006). Spatial autocorrelation analyses are individual-based rather than population-based, and therefore they are not influenced by the subjective pooling of samples. genalex uses pairwise geographical and pairwise squared genetic distance matrices to calculate an autocorrelation coefficient r for a set of distance classes specified by the user (Smouse & Peakall 1999; Peakall, Ruibal & Lindenmayer 2003). The autocorrelation coefficient provides a measure of the genetic similarity between pairs of individuals whose geographical separation falls within the specified distance class. We used the total pairwise genetic distance matrix (i.e. the matrix obtained from the sum of the matrices obtained for each locus) as long as no evidence of linkage disequilibrium between each pair of loci was detected. The linear pairwise geographical distance matrix was calculated from X- and Y-coordinates of each of the 35 colonies sampled in the Ebro Valley. Since most re-sightings concerning dispersal occurred within a radius of 10 km (Fig. 1), we chose a set of variable distance classes for the analysis with a minimum distance class of 10 km. The calculated autocorrelation coefficients r were then plotted as a function of distance. Following Peakall *et al*. (2003), tests for statistical significance were performed using random permutations (N = 999) and bootstrap estimates of r (N = 999).

The software structure 2·2 (Pritchard, Stephens & Donnelly 2000) was used to test for the presence of genetically distinct clusters in Western Europe (N = 432 individuals). We did not use any prior information about the origin of the individuals and we assumed correlated allele frequencies and the admixture model. Ten simulations were performed for each of the K values ranging from 1 to 6 (i.e. number of putatively different genetic clusters) and probability values of the data, i.e. lnPr(X/K), were plotted. Analyses were carried out with 100 000 iterations, following a burn-in period of 10 000 iterations. We also calculated the traditional estimate of genetic differentiation F_{ST} to investigate population differentiation in Western Europe. The distributions of allele frequencies between the four geographically distinct populations (i.e. Ebro Valley, Spanish core area, Portugal and France, Fig. 2) were compared using the software genetix 4·04 (Belkhir *et al*. 1996–2002). The significance of F_{ST} pairwise comparisons was given by a P value calculated using 10 000 random permutation tests that was further adjusted according to sequential Bonferroni corrections for multiple tests (Rice 1989). Previously, we tested whether the Ebro Valley and the Spanish core area could be considered as large random breeding units attending to their conformity to Hardy–Weinberg equilibrium. In addition, the spatial autocorrelation analysis will check for local genetic structuring in the Ebro Valley and pairwise F_{ST} values between peripheral and centrally sampled localities will test for population differentiation within the Spanish core area. Even though structure results suggest a genetically uniform population (i.e. K = 1), testing for differences in allele frequencies between geographically distinct populations can be more powerful than structure analyses when dealing with low levels of genetic differentiation (see software documentation in http://pritch.bsd.uchicago.edu/software/structure22/readme.pdf).

Results

LOCI PROPERTIES

Overall, 105 alleles were detected across nine microsatellite markers and 432 genotyped birds. Loci properties (i.e. number of alleles per locus, range size and average heterozygosities) are summarized in Table 2. No significant evidence of linkage disequilibrium was observed in any pair of loci analysed. Only locus Fp107 departed significantly from Hardy–Weinberg expectations. This locus consistently showed heterozygosity deficits that must be related to the presence of null alleles (see Alcaide *et al*. 2005). Since null alleles may violate several assumptions of the genetic methods we intended to apply, locus Fp107 was removed from further analysis.

GENETIC STRUCTURE IN THE EBRO VALLEY

The spatial autocorrelation analysis within the Ebro Valley population revealed a lack of fine-scale spatial patterns of genetic differentiation. The autocorrelogram plotted by genalex 6·0 (Fig. 3) showed that no genetic autocorrelation

Table 2. Microsatellite diversity at each of the four geographically distinct populations investigated in this study. Observed (H_O) vs. expected heterozygosities (H_E) and estimates of allele richness (A_r) based on 25 individuals are indicated

Population	H_E	H_O	A_r (no. of alleles per locus)
Ebro Valley	0·65	0·64	6·99
Spanish Core Area	0·65	0·65	7·5
Portugal	0·66	0·65	7
France	0·60	0·60	6·22

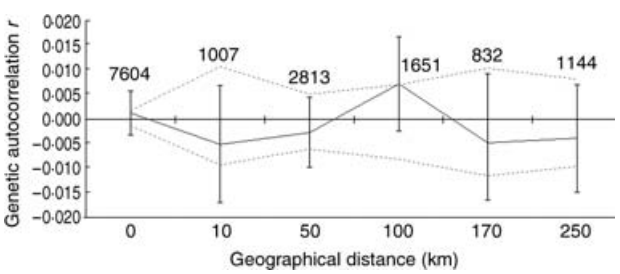

Fig. 3. Correlogram plot of the degree of genetic similarity between lesser kestrel nestlings as a function of geographical distance in the Ebro Valley population (N = 174). The permuted 95% confidence interval (dashed lines) and the bootstrapped 95% confidence error (bars) are also shown. The number of pairwise combinations within each distance class is presented above the plotted values.

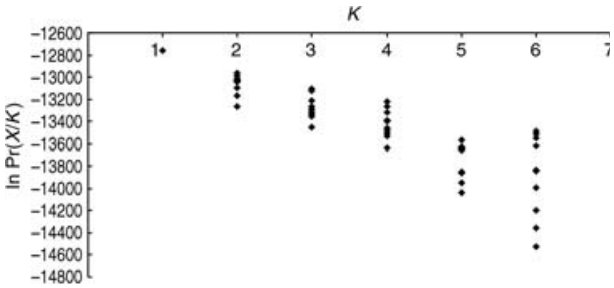

Fig. 4. Bayesian clustering analysis of 432 lesser kestrels in the Iberian Peninsula. For each value of K (i.e. number of putatively different genetic clusters tested), 10 simulations were carried out to obtain the probability of the data (y-axis).

Table 3. F_{ST} pairwise values (above diagonal) between the four geographically distinct populations of Western Europe (see Fig. 2). Significant values after Bonferroni corrections for multiple tests are outlined in bold. Non-Bonferroni corrected P values are given below the diagonal

	Ebro Valley	Spanish core area	Portugal	France
Ebro Valley (N = 174)		**0·003**	0·005	**0·012**
Spanish core area (N = 207)	0·002		0·004	**0·016**
Portugal (N = 25)	0·08	0·011		**0·027**
France (N = 26)	0·002	< 0·001	< 0·001	

coefficient was significantly different from zero at any distance class. In addition, there is no evidence of decreased genetic similarity in nestlings as a function of geographical distance. The Ebro Valley population also seems to behave as a large random mating population as suggested by its conformity to Hardy–Weinberg proportions (H_E: 0·64, H_O: 0·63; Bonferroni corrected P value > 0·05).

GENETIC STRUCTURE IN WESTERN EUROPE

The Bayesian model-based clustering method implemented in structure suggested panmixia (i.e. K = 1) as the most likely scenario in Western Europe (see Fig. 4). In addition, we did not find genetic differentiation within the Spanish core area as revealed by the lack of statistically significant F_{ST} values between peripheral and central localities (all F_{ST} values < 0·006, Bonferroni corrected P values > 0·05). Conformity to Hardy–Weinberg expectations in the Spanish core area (H_E: 0·65, H_O: 0·65, Bonferroni corrected P value > 0·05) also supports its consideration as a large random breeding unit. Both the sampled populations from France and Portugal fitted to Hardy–Weinberg equilibrium as well (France, H_E: 0·60 vs. H_O: 0·60, Bonferroni corrected P value > 0·05; Portugal, H_E: 0·66 vs. H_O: 0·65, Bonferroni corrected P value > 0·05).

Even though we did not find local genetic structure in the Ebro Valley population and structure results suggested a genetically uniform population of lesser kestrels in Western Europe, our F_{ST} analysis between the four geographically distinct populations revealed weak but statistically significant population differentiation. Genetic divergence seemed to be stronger and biologically relevant in the case of the geographically isolated breeding population of Southern France. In addition, genetic differentiation with respect to France appeared to increase as a function of geographical distance (Table 3).

Discussion

Our fine-scale analysis of population structure in lesser kestrels has failed to detect increased genetic similarity between those nestlings hatched at short distances despite the high philopatry rates and restricted dispersal documented by capture–recapture analyses (Negro *et al*. 1997; Serrano *et al*. 2001, 2003; Serrano & Tella 2003; Ortego *et al*. 2008; Serrano, Carrete & Tella 2008). This lack of local genetic structure among breeding colonies supports previous genetic patterns found in other philopatric, socially monogamous and colonially breeding seabirds (e.g. Austin, White & Ovenden 1994; Abbott & Double 2003; Van Bekkum *et al*. 2006; Nims *et al*. 2007). Therefore, these findings would to some extent challenge traditional assumptions associating philopatry with the emergence of genetic structuring (Greenwood 1980; Sugg *et al*. 1996). A recent study by Ortego and co-workers (2008) has however reported a weak but significant positive autocorrelation between breeding individuals (N = 381) at the colony level that we failed to find in nestlings (N = 174). These authors argued that this fact must be due to the sharing of the same colony by some related individuals. For instance, several siblings can recruit as breeders into the same colony where they were born. While this direct kinship relationships (i.e. sibling–sibling, parent–sibling) may inflate genetic similarity indexes, the degree of relatedness between nestlings when only one individual per nest is sampled (i.e. cousin–cousin) could comparably decrease if individuals avoid mating with relatives. Furthermore, this potential difference in the sampling situation could be exacerbated by differences in natal dispersal patterns between both populations (59% in La Mancha vs. 83% in the Ebro valley, Serrano *et al*. 2003; Ortego *et al*. 2008). Finally, Ortego and co-workers amplified four microsatellite markers departing from Hardy–Weinberg equilibirum, with observed heterozygosities below expectations (e.g. Ortego *et al*. 2007a). Particularly, they provided statistical support for significant heterozygosity deficits in two markers (Loci Fana2-14 and Fp86-2, Ortego *et al*. 2007b). An excess of homozygous genotypes in the population, probably due to the amplification of null alleles, may also artificially inflate genetic similarity indexes between relatives. All loci that we used for the analyses presented in this study fitted to Hardy–Weinberg expectations, thus avoiding this potential bias.

Human-induced habitat perturbations have been shown to generate patterns of genetic differentiation in avian populations (e.g. Caizergues *et al*. 2003; Martínez-Cruz, Godoy & Negro 2004). The finding of panmixia in the Ebro Valley however suggests that population subdivision at relatively small geographical scales are not sufficient enough to restrict

dispersal capabilities of lesser kestrels, as it has been suggested through capture–recapture analyses (Serrano & Tella 2003). We are confident that patterns of dispersal derived from capture–recapture data are scarcely biased by long-distance dispersers in the intensively monitored Ebro Valley population, given that (i) annual survival probabilities between fledgling and first-breeding as estimated with capture–recapture models were about 50–60% (D. Serrano, unpublished data), similar to the highest estimates described for the species (Prugnolle *et al*. 2003), and hence hardly unbiased by permanent dispersal; (ii) maximum dispersal distance within our population (136 km) was much shorter than maximum distance between colonies (210 km) (Serrano *et al*. 2003), and (iii) only one local bird was known to recruit in the surrounding populations (see below), which were subject to thorough monitoring programmes. Thus, our combined approach seems to indicate that a few long-distance dispersal events are enough to connect genetically distant patches, dilute genetic signatures, and homogenize allele frequencies, as previously suggested in the literature (Mills & Allendorf 1996; Vucetich & Waite 2000). Furthermore, this population could have maintained or reached effective population sizes large enough to prevent the development of local genetic structure through genetic drift despite an initially low number of breeding pairs (see Serrano & Tella 2007 for details).

Apart from spatial population fragmentation, the emergence of genetic structure in avian populations at relatively small geographical scales has been related to complex mating systems such as those displayed by lekking (e.g. Höglund & Shorey 2003; Bouzat & Johnson 2004) or cooperatively breeding species (e.g. Woxvold, Adcock & Mulder 2006), in which one sex is much more philopatric than the other. In fact, several avian studies have related fine-scale spatial patterns of genetic differentiation to pronounced sex-biased dispersal (e.g. Fowler 2005; Double *et al*. 2005; McKinnon, Gilchrist & Scribner 2006; Temple *et al*. 2006). Lesser kestrels are socially monogamous (see Tella *et al*. 1996 for a rare exception) and do not exhibit cooperative breeding strategies. Although our sampling protocol did not allow the detection of sex-specific genetic structuring, it is known that both males and females are highly philopatric, with natal dispersal distances greatly overlapping for the two sexes (Negro *et al*. 1997; Serrano *et al*, 2003). Moreover, capture–recapture studies have shown that the presence of the parent or a sibling of the opposite sex had no effect on whether or not first breeders returned to breed to the natal colony, either in the Guadalquivir (Negro *et al*. 1997) or in the Ebro Valley population (Serrano *et al*. 2003). Thus, genetic structure in lesser kestrels is not expected to emerge as a consequence of complex reproductive behaviours or pronounced sex-biased dispersal patterns.

Although philopatry does not seem to have generated local genetic structure, restricted dispersal could have enhanced the effect of population fragmentation at a larger geographical scale. The Bayesian clustering method implemented in structure, however, did not provide evidence for the existence of genetically distinct clusters once we scaled-up our study area to the entire distribution of the species in Western Europe. In the well-studied Ebro Valley population, re-encounters of ringed birds suggest that long-distance dispersal is anecdotal, with three immigrant birds banded elsewhere, two of them banded in central Spain and another one in France (J.L. Tella, D. Serrano & E. Ursúa, unpublished data), and one male ringed as a nestling in the Ebro Valley that recruited as a breeding adult in the reintroduced population of eastern Spain, 300 km away (M. Alberdi, personal communication). As mentioned above, these few migrants may be sufficient to result in the development of low genetic subdivision, although at this scale the importance of long-distance dispersal events may have been underestimated by capture–recapture methods (see for instance Koenig & Dickinson 2004). Nonetheless, F_{ST} comparisons revealed weak but statistically significant population differentiation among the four geographically distinct populations of Western Europe. This finding would underscore the limitations of structure to infer population structure when F_{ST} values are low (see also Latch *et al*. 2006).

At the very least, genetic divergence was shown to be consistent and biologically relevant in the case of the isolated French population. This population showed increased genetic divergence as a function of distance (Table 3), a fact that supports the isolation-by-distance genetic patterns suggested at both local (Ortego *et al*. 2008) and continental scales (Alcaide *et al*. 2008), and which is consistent with the distance-dependent model of dispersal derived from capture–recapture analyses as well (Negro *et al*. 1997; Serrano *et al*. 2003; Serrano & Tella 2003; Ortego *et al*. 2008). Restricted gene flow in the case of the French population could also be a consequence of limited immigration resulting from the low conspecific attraction exerted by small breeding populations (see Serrano & Tella 2003; Serrano *et al*. 2004).

In conclusion, our genetic data suggest a lack of fine-scale genetic structuring in lesser kestrel populations, with low genetic differentiation at a larger geographical scale. Ringing records, in turn, indicate that most individuals were philopatric to the natal colony or dispersed short distances along their life span. In this respect, our integrative approach suggests that rare long-distance dispersal events seem sufficient to override the predictions derived from high philopatry rates in birds. This fact highlights the benefits of combining traditional capture–recapture with modern genetic methods in order to improve our understanding of dispersal and connectivity in animal (meta)populations. One could argue that the straightforward genetic approach shows enough gene flow to maintain panmictic populations that the more laborious capture–recapture methodology is unable to detect, thus casting doubt on the utility of the second approach. However, the first approach overlooks the actual limited dispersal ability of the species, which might compromise the viability of small and isolated local populations even at small spatial scales (Serrano & Tella 2003). Whereas our results may therefore lessen to some extent the genetic concerns derived from habitat fragmentation in species with apparently limited dispersal abilities, this study also raises concerns about increased genetic divergence in small and geographically distant populations. Determining

minimum thresholds above which dispersal capabilities cannot counteract the harmful genetic and demographic effects of habitat fragmentation is a laborious species- and even population-specific task, but of crucial importance to effectively manage spatially structured populations.

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References

Abbott, C.L. & Double, M.C. (2003) Genetic structure, conservation genetics and evidence of speciation by range expansion in shy and white-capped albatrosses. *Molecular Ecology*, **12**, 2953–2962.

Alcaide, M., Negro, J.J., Serrano, D., Tella, J.L. & Rodriguez, C. (2005) Extra-pair paternity in the lesser kestrel *Falco naumanni*: a re-evaluation using microsatellite markers. *Ibis*, **147**, 608–611.

Alcaide, M., Edwards, S.V., Negro, J.J., Serrano, D. & Tella, J.L. (2008) Extensive polymorphism and geographical variation at a positively selected MHC class II B gene of the lesser kestrel (*Falco naumanni*). *Molecular Ecology*, **17**, 2652–2665.

Alcázar, R. & Henriques, I. (2006) *Recuperaçao do Peneireiro-das-torres* (Falco naumanni) *em Portugal*. Relatório Técnico Final do Projecto Peneireiro-das-torres, LPN Portugal.

Arsenault, D.P., Stacey, P.B. & Hoelzer, G.A. (2002) No extra-pair fertilization in flammulated owls despite aggregated nesting. *The Condor*, **104**, 197–201

Austin, J.J., White, R.W.G. & Ovenden, J.R. (1994) Population-genetic structure of a philopatric, colonially nesting seabird, the short-tailed shearwater (*Puffinus tenuirostris*). *Auk*, **111**, 70–79.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. (1996–2002) *GENETIX 4.04 Logiciel sous Windows TM, pour la Génétique des Populations*. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Berry, O., Tocher, M.D. & Sarre, S.D. (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561

Biber, J.P. (1990) *Action Plan for the Conservation of Western Lesser Kestrel* (Falco naumanni) *Populations*. International Council for Bird Preservation (Study Report 41), Cambridge, UK.

BirdLife International (2007) *Species Factsheets*. Downloaded from http://www.birdlife.org on 03/01/2007.

Bossar, J.L. & Prowell, D.P. (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology & Evolution*, **13**, 202–206.

Bouzat, J.L. & Johnson, K. (2004) Genetic structure among closely spaced leks in a peripheral population of lesser prairie chickens. *Molecular Ecology*, **13**, 499–505.

Caizergues, A., Rätti, O., Helle, P., Rotelli, L., Ellison, L. & Rasplus, J.-Y. (2003) Population genetic structure of male black grouse (*Tetrao tetrix* L.) in fragmented vs. continuous landscapes. *Molecular Ecology*, **12**, 2297–2305.

Cheylan, G. (1991) Le faucon crécerellette en France: statut actuel et régression. *Faune Provence*, **12**, 45–49.

Clobert, J., Danchin, E., Dhondt, A.A. & Nichols, J.D. (2001) *Dispersal*. Oxford University Press, New York.

Cramp, S. & Simmons, K.E.L. (1980) *The Birds of the Western Palearctic*, Vol. 2. Oxford University Press, Oxford, UK.

Double, M.C., Peakall, R., Beck, N.R. & Cockburn, A. (2005) Dispersal, philopatry and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). *Evolution*, **59**, 625–635.

Fowler, A.C. (2005) Fine-scale spatial structuring in cackling Canada geese related to reproductive performance and breeding philopatry. *Animal Behaviour*, **69**, 973–981.

Frankham, R., Ballou, J.D. & Briscoe, D.A. (2002) *Introduction to Conservation Genetics*. Cambridge University Press, New York.

Gemmell, N.J. & Akiyama, S. (1996) An efficient method for the extraction of DNA from vertebrate tissues. *Trends in Genetics*, **12**, 338–339.

Gompper, M.E., Gittleman, J.L. & Wayne, R.K. (1998) Dispersal, philopatry, and genetic relatedness in a social carnivore: comparing males and females. *Molecular Ecology*, **7**, 157–163.

Greenwood, P.J. (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Greenwood, P.J. & Harvey, P.H. (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics*, **13**, 1–21.

Groombridge, J.J., Jones, C.G., Bruford, M.W. & Nichols, R.A. (2000) 'Ghost' alleles of the Mauritius kestrel. *Nature*, **403**, 616.

Hansson, B., Bensch, S. & Hasselquist, D. (2004) Lifetime fitness of short-and long distance dispersing great reed warblers. *Evolution*, **58**, 2546–2557.

Höglund, J. & Shorey, L. (2003) Local genetic structure in a white-bearded manakin population. *Molecular Ecology*, **12**, 2457–2463

Koenig, W.D. & Dickinson, J.L. (2004) Introduction. *Ecology and Evolution of Cooperative Breeding in Birds* (eds W.D. Koenig & J.L. Dickinson). Cambridge University Press, Cambridge, UK.

Koenig, W.D., Van Vuren, D., & Hooge, P.H. (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology & Evolution*, **11**, 514–517.

Korpimäki, E., Lahti, K., May, C.A., Parkin, D.T., Powell, G.B., Tolonen, P. & Wetton, J. (1996) Copulatory behaviour and paternity determined by DNA fingerprinting in kestrels: effects of cyclic food abundance. *Animal Behaviour*, **51**, 945–955.

Latch, E.K., Dharmarajan, G., Glaubitz, J.C. & Rhodes, O.E. (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295–302.

Manel, S., Gaggioti, O.E. & Waples, R.S. (2005) Assignment methods: matching biological questions with appropriate methods. *Trends in Ecology & Evolution*, **20**, 136–142.

Martínez-Cruz, B., Godoy, J.A. & Negro, J.J. (2004) Population genetics after fragmentation: the case of the Spanish imperial eagle *Aquila adalberti*. *Molecular Ecology*, **13**, 2243–2255.

McKinnon, L., Gilchrist, H.G. & Scribner, K.T. (2006) Genetic evidence for kin-based female social structure in common eiders (*Somateria mollissima*). *Behavioural Ecology*, **17**, 614–621.

Mills, S.L. & Allendorf, F.W. (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10**, 1509–1518.

Negro, J.J. (1997) *Lesser kestrel Falco naumanni*. BWP [Birds of the Western Palearctic] Update, **1**, 49–56.

Negro, J.J., Hiraldo, F. & Donazar, J.A. (1997) Causes of natal dispersal in the lesser kestrel: inbreeding avoidance or resource competition? *Journal of Animal Ecology*, **66**, 640–648.

Nesje, M., Roed, K.H., Lifjeld, J.T., Lindberg, P. & Steens, O.F. (2000) Genetic relationship in the peregrine falcon (*Falco peregrinus*) analysed by microsatellite DNA markers. *Molecular Ecology*, **9**, 53–60.

Nims, B.D., Hernán Vargas, F., Merkel, J. & Parker P.G. (2007) Low genetic diversity and lack of genetic structure in the endangered Galápagos penguin (*Spheniscus mendilicus*). *Conservation Genetics*, doi: 10.1007/s10592-007-9465-1.

Ortego, J., Calabuig, G., Cordero, P.J. & Aparicio, J.M. (2007a) Egg production and individual genetic diversity in lesser kestrels. *Molecular Ecology*, **16**, 2383–2392.

Ortego, J., González, E.G., Sánchez-Barbudo, I., Aparicio, J.M. & Cordero, P.J. (2007b) Novel highly polymorphic loci and cross-amplified microsatellites for the lesser kestrel *Falco naumanni*. *Ardeola*, **54**, 101–108.

Ortego, J., Calabuig, G, Aparicio, J.M. & Cordero, P.J. (2008) Genetic consequences of natal dispersal in the colonial lesser kestrel. *Molecular Ecology*, **17**, 2051–2057.

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C. (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Peacock, M.M. & Ray, C. (2001) Dispersal in pikas (*Ochotoma princes*): combining genetic and demographic approaches to reveal spatial and temporal patterns. *Dispersal* (eds Clobert, J., Danchin, E., Dhondt, A.A. and Nichols, J.D.), pp. 43–56. Oxford University Press, Oxford, UK.

Peakall, R. & Smouse, P.E. (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Peakall, R., Ruibal, M. & Lindenmayer, D.B. (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, **57**, 1182–1195.

Pilard, P. & Brunn, L. (1998) La reproduction du faucon crécerellette Falco naumanni en Crau et le bilan des aménagements de sites de nidification en faveur de celte population. *Faune Provence*, **19**, 43–50.

Pritchard, J.K., Stephens, P. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Prugnolle, F., Pilard, P., Brun, L. & Tavecchia, G. (2003) First-year and adult survival of the endangered lesser kestrel *Falco naumanni* in southern France. *Bird Study*, **50**, 68–72.

Raymond, M. & Rousset, F. (1995) genepop (version 1·2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice, W.R. (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Senar, J.C., Borras, T., Cabrera, J., Cabrera, T., & Björklund, M. (2006) Local differentiation in the presence of gene flow in the citril finch Serinus citrinella. *Biology Letters*, **2**, 85–87.

Serrano, D. & Tella, J.L. (2003) Dispersal within a spatially structured population of lesser kestrel: the role of spatial isolation and conspecific attraction. *Journal of Animal Ecology*, **72**, 400–410.

Serrano, D., Oro, D., Ursúa, E. & Tella, J.L. (2005) Colony size selection determines adult survival and dispersal preferences: Allee effects in a colonial bird. *American Naturalist*, **166**, 22–31.

Serrano, D. & Tella, J.L. (2007) The role of despotism and heritability in determining settlement patterns in the colonial lesser kestrel. *The American Naturalist*, **169**, E53–E67.

Serrano, D., Tella, J.L., Forero, M.G. & Donázar, J.A. (2001) Factors affecting breeding dispersal in the facultatively colonial lesser kestrel: individual experience vs. conspecifics cues. *Journal of Animal Ecology*, **70**, 568–578.

Serrano, D., Tella, J.L., Donázar, J.A. & Pomarol, M. (2003) Social and individual features affecting natal dispersal in the colonial lesser kestrel. *Ecology*, **84**, 3044–3054.

Serrano, D., Forero, M.G., Donázar, J.A. & Tella, J.L. (2004) Dispersal and social attraction affect colony selection and dynamics of lesser kestrels. *Ecology*, **85**, 3438–3447.

Serrano, D., Carrete, M. & Tella, J.L. (2008). Describing dispersal under habitat constraints: a randomization approach in lesser kestrels. *Basic and Applied Ecology*, **9**, 771–778.

Smouse, P.E. & Peakall, R. (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.

Sugg, D.W., Chesser, F.S., Dobson, J.L. & Hoogland, J.L. (1996) Population genetics meets behavioural ecology. *Trends in Ecology & Evolution*, **11**, 338–342.

Tella, J.L., Negro, J.J., Villarroel, M., Kuhnlein, U., Hiraldo, F., Donázar, J.A. & Bird, D.M. (1996) DNA fingerprinting reveals polygyny in the Lesser kestrel (Falco naumanni). *The Auk*, **113**, 262–265.

Temple H.J., Hoffman J.I. & Amos W. (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the white-breasted thrasher *Ramphocinclus brachyurus*. *Molecular Ecology*, **15**, 3449–3458.

Van Bekkum, M., Sagar, P.M., Stahl, J.C. & Chambers, G.K. (2006) Natal philopatry does not lead to population genetic differentiation in Buller's albatross (*Thalassarche bulleri bulleri*) *Molecular Ecology*, **15**, 73–79.

Vilà, C., Sundqvist, A.-K., Flagstad, Ø., Seddon, J., Björnerfeldt, S., Kojola, I., Casulli, A., Sand, H., Wabakken, P. & Ellegren, H. (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 91–97.

Vucetich, J.A. & Waite, T.A. (2000) Is one migrant per generation sufficient for the genetic management of fluctuating populations? *Animal Conservation*, **3**, 261–266.

Whitlock, M.C. & McCauley, D.E. (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity*, **82**, 117–125.

Woxvold, I.A., Adcock, G.J. & Mulder R.A. (2006). Fine-scale genetic structure and dispersal in cooperatively breeding apostlebirds. *Molecular Ecology*, **15**, 3139–3146.

Young, A. & Clarke, G. (eds) *Genetics, Demography and the Viability of Fragmented Populations*. Cambridge University Press, Cambridge, UK.

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