ORIGINAL ARTICLE

Population fragmentation leads to isolation by distance but not genetic impoverishment in the philopatric Lesser Kestrel: a comparison with the widespread and sympatric Eurasian Kestrel

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Population fragmentation is a widespread phenomenon usually associated with human activity. As a result of habitat transformation, the philopatric and steppe-specialist Lesser Kestrel *Falco naumanni* underwent a severe population decline during the last century that increased population fragmentation throughout its breeding range. In contrast, the ubiquitous Eurasian Kestrel *Falco tinnunculus* did not suffer such adverse effects, its breeding range still remaining rather continuous. Using microsatellites, we tested the effects of population fragmentation on large-scale spatial patterns of genetic differentiation and diversity by comparing these two sympatric and phylogenetically related species. Our results suggest that habitat fragmentation has increased genetic differentiation between Lesser Kestrel populations, following an isolation-by-distance pattern, while the population of Eurasian Kestrels is panmictic. Contrary to expectations, we did not detect significant evidence of reduced genetic variation or increased inbreeding in Lesser Kestrels. Although this study reports genetic differentiation in a species that has potential for long-distance dispersal but philopatry-limited gene flow, large enough effective population sizes and migration may have been sufficient to mitigate genetic depauperation. A serious reduction of genetic diversity in Lesser Kestrels would, therefore, only be expected after severe population bottlenecks following extreme geographic isolation.
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Introduction

Human activities transform the natural habitats of many species. Population fragmentation often leads to overall reductions in population sizes and diminishes connectivity among habitat patches. Although population fragmentation increases extinction risks because of deterministic and stochastic effects on demographic parameters, restricted gene flow may jeopardize long-term persistence of populations due to inbreeding depression and loss of genetic diversity. Both demographic and genetic impacts of population fragmentation are believed to depend on the number, size and spatial distribution of populations as well as on time since fragmentation. In this regard, dispersal and associated gene flow appears one of the most critical factors influencing the genetic structure and demography of fragmented populations (for example, Young and Clarke, 2000; Frankham *et al.*, 2002). However, restricted gene flow and the subsequent emergence of genetic

structuring is not only the result of physical barriers or anatomical impediments to long-distance movement. Natal and breeding philopatry (that is, the tendency of individuals to breed close to their birthplace or their previous breeding territory) is expected to enhance the effects of habitat fragmentation (for example, Greenwood, 1980). Genetic differentiation among fragments is hence expected to be inversely correlated with the dispersal ability of the species.

In spite of all these considerations, there is not necessarily a direct association between the spatial distribution of populations and the spatial distribution of genetic diversity (for example, Dannewitz *et al.*, 2005; Jones *et al.*, 2007; Koopman *et al.*, 2007). Combined demographic and genetic investigations are therefore being encouraged to rigorously evaluate the consequences of population fragmentation (for example, Koenig and Dickinson, 2004). In this respect, elucidating the demographic and ecological factors that determine the distribution of genetic variation at different scales has become fundamental to research in conservation and evolutionary biology. Polymorphic molecular markers and powerful statistical methods have allowed the investigation of the spatial distribution of genetic variation in fragmented populations and provided a measure of population connectivity. Such approaches,

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combined with life-history and demographic information, have consistently provided relevant data to underpin conservation and management initiatives aimed at preserving the genetic diversity of endangered species (for example, Caizergues *et al.*, 2003; Martínez-Cruz *et al.*, 2004; Hansson and Richardson, 2005; Koopman *et al.*, 2007).

Studies of genetic structure and diversity in birds of prey are accumulating due to an emerging concern about the threats derived from population fragmentation and habitat alteration in this charismatic avian group (for example, Godoy *et al.*, 2004; Martínez-Cruz *et al.*, 2004; Helbig *et al.*, 2005; Brown *et al.*, 2007; Cadahía *et al.*, 2007; Hailer *et al.*, 2007; Nittinger *et al.*, 2007). Birds of prey typically have small populations, extended distributional ranges and they usually have long-distance dispersal capabilities. Although raptor populations tend to be poorly structured (see references above), habitat fragmentation could potentially increase genetic divergence among populations and reduced population size would initiate a loss of genetic variation. In this study, we employed polymorphic microsatellites to assess the influence of population fragmentation on genetic diversity and large-scale (continental) spatial patterns of genetic differentiation in two phylogenetically related and sympatric birds of prey, the Lesser Kestrel *Falco naumanni* and the Eurasian Kestrel *Falco tinnunculus*. Both species breed in Eurasia, a continental mass with a broad tradition of human-induced landscape transformations, which have generated serious threats for the conservation of many species (Goriup and Batten, 1990; McNeely, 1994). Although the Lesser Kestrel is a specialist falcon inhabiting steppe and pseudosteppe ecosystems (Cramp and Simmons, 1980), the Eurasian Kestrel is considered a truly cosmopolitan falcon that can live in most open-country environments (Village, 1990). Open habitats in Europe have increased due to agriculture and clear-cutting of forests, a fact that may explain why the breeding range of the Eurasian Kestrel has not been decisively affected by human activities. In contrast, Lesser Kestrels have experienced a well-documented population decline during the twentieth century that is mostly explained by human perturbations, such as the substitution of traditional agricultural practices by intensive agriculture and irrigated crops that reduce foraging habitats (Tella *et al.*, 1998; Ursúa *et al.*, 2005). Such a dramatic population regression led to the extirpation or disappearance of the Lesser Kestrel from several European countries (Biber, 1990). It consequently has a patchier distributional breeding range as compared with its generalist counterpart (Figure 1). In addition, long-term and extensive ringing studies of Lesser Kestrels in Spain have documented high natal and breeding philopatry as well as a negative association between effective dispersal and geographical distance (Negro *et al.*, 1997; Serrano *et al.*, 2001, 2003, 2008). Conversely, Eurasian Kestrels have shown a low philopatry and frequent effective long-distance dispersals in populations from Northern and Western Europe (Korpimäki, 1988; Village, 1990; Korpimäki *et al.*, 2006; Vasko, 2007), although preliminary data from a Spanish population suggest higher philopatry rates in Southern Europe (JA Fargallo, personal communication).

Hence, the main question that this study will address is whether habitat alteration has resulted in population
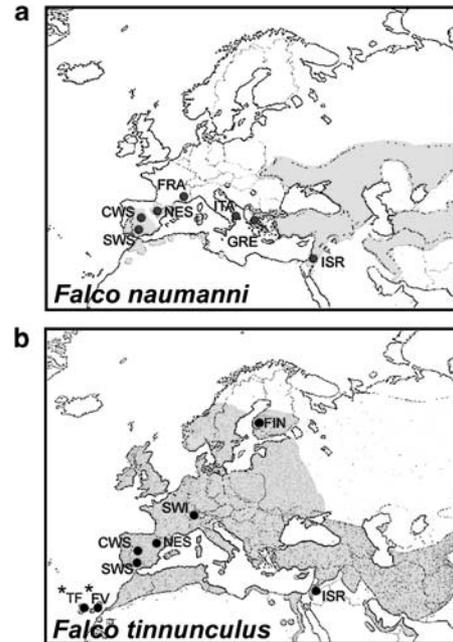

Figure 1 Breeding distributional ranges (grey areas) of Lesser (**a**) and Eurasian (**b**) Kestrels across the Western Palearctic. Populations analysed in this study are indicated by black dots. Lesser Kestrels were sampled from southwestern Spain (SWS), central-western Spain (CWS), northeastern Spain (NES), France (FRA), Italy (ITA), Israel (ISR) and Kazakhstan (KAZ). The continental subspecies of the Eurasian Kestrel was sampled from SWS, CWS, NES, Switzerland (SWI), Finland (FIN) and ISR. In addition, two subspecies of the Eurasian Kestrel inhabiting the Canary Islands (indicated by asterisks) were sampled (FV for *Falco tinnunculus dacotiae* and TF for *Falco tinnunculus canariensis*).

differentiation and loss of genetic diversity in the highly philopatric Lesser Kestrel compared with the widely distributed and highly dispersive Eurasian Kestrel. The suitability of the genetic methods we used here was tested by means of additional analyses of two insular subspecies of the Eurasian Kestrel inhabiting the Canary Islands. We expected the populations of these subspecies to hold comparably lower levels of genetic variation because of the well-documented effects of insularity on demography and genetic diversity (for example, Bollmer *et al.*, 2005).

Materials and methods

Study species and populations
The Lesser Kestrel is a small trans-Saharian migratory falcon whose breeding range covers mid-latitude and low elevations of Eurasia (Cramp and Simmons, 1980). This colonial falcon originally occupied small cliffs surrounded by natural steppes (Tella *et al.*, 2004), but most pairs breed nowadays in human structures surrounded by traditional agricultural land. The Eurasian Kestrel is a sedentary or partially migratory falcon of slightly larger size that is widespread in Eurasia, normally showing a territorial breeding behaviour (Cramp and Simmons, 1980). In Europe, the estimated population size of Lesser Kestrels is about 25 000–42 000 breeding pairs, whereas that of Eurasian Kestrels is about 300 000–500 000 breeding pairs. We analysed breeding

populations of the Lesser Kestrel in southwestern Spain, central-western Spain, northeastern Spain, France, Italy, Greece and Israel (see Figure 1a). The continental subspecies of the Eurasian Kestrel (*Falco tinnunculus tinnunculus*) was sampled in southwestern Spain, central-western Spain, northeastern Spain, Switzerland, Finland and Israel (see Figure 1b). Two insular subspecies of the Eurasian Kestrel inhabiting the Canary Islands, *Falco tinnunculus canariensis* and *Falco tinnunculus dacotiae* (see Figure 1b), were also investigated to provide comparative data. Estimated population sizes are about 400–500 breeding pairs for *F. t. dacotiae* and less than 4000 breeding pairs for *F. t. canariensis* (Madroño *et al.*, 2004).

The majority of sampled individuals (>90%) were nestlings, and we only analysed one individual per brood to minimize problems associated with close relatedness. Extra-pair paternity in Lesser and Eurasian Kestrels has shown to be rare (below 7.5% of nestlings, see Korpimäki *et al.* (1996) and Alcaide *et al.* (2005) for details), and thus, the probability for adult males to raise their own offspring is high. Estimated population sizes of the geographically distinct populations of Lesser Kestrels investigated in this study are shown in Table 1. The number of Lesser and Eurasian Kestrels sampled at each location is shown in 3 and 4, respectively.

DNA isolation and microsatellite genotyping

About 100 µl of blood preserved in 96% ethanol or growing feathers that were pulled from the birds' dorsal plumage were digested by incubation with proteinase K for at least 3 h. DNA purification was carried out by using 5 M LiCl organic extraction method with chloroform/isoamylic alcohol (24:1) and further DNA precipitation using absolute ethanol. Pellets obtained were dried and washed twice with 70% ethanol, and later stored at $-20\,^{\circ}\mathrm{C}$ in 0.1 ml of TE buffer. We amplified seven microsatellites that were isolated originally in the peregrine falcon *Falco peregrinus* by Nesje *et al.* (2000) (*Fp5*, *Fp13*, *Fp31*, *Fp46-1*, *Fp79-4*, *Fp89* and *Fp107*). In addition, we designed two sets of primers flanking two microsatellite sequences also isolated in the peregrine falcon that were 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 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 $94\,^{\circ}\mathrm{C}$, 40 s at

Table 1 Estimated population sizes of Lesser Kestrels sampled for this study

Location	Code	Population size (breeding pairs)
Spanish core area	SWS and CWS	12 000–19 000
Ebro Valley	NES	1000
France	FRA	<100
Italy	ITA	3640–3840
Greece	GRE	2000–3480
Israel	ISR	<1000

Data were taken from BirdLife International (2007), Prugnolle *et al.* (2003) and Liven-Schulman *et al.* (2004). See Figure 1 for geographic locations.

$55\,^{\circ}\mathrm{C}$, 40 s at $72\,^{\circ}\mathrm{C}$ and finally, 4 min at $72\,^{\circ}\mathrm{C}$. Each 11 µl reaction contained 0.2 U of Taq polymerase (Bioline, London, UK), $1\times$ PCR manufacturer-supplied buffer, 1.5 mM MgCl$_2$, 0.02% gelatine (Amersham Life Sciences, Buckinghamshire, UK), 0.12 mM of each dNTP, 5 pmol of each primer and, approximately, 10 ng of genomic DNA. Forward primers were 5′-end labelled with HEX, NED or 6-FAM. Amplified fragments were resolved on an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

Genetic analyses

Polymorphism statistics at each microsatellite marker (that is, the number of alleles and range size of the amplified fragments) were calculated using the programme Genetix 4.04 (Belkhir *et al.*, 1996–2004). Conformity to Hardy–Weinberg equilibrium was analysed through GENEPOP (Raymond and Rousset, 1995), using a single locus and a global multilocus test for heterozygosity deficit or excess by the Markov Chain Method (Raymond and Rousset, 1995).

We employed the software STRUCTURE 2.2 (Pritchard *et al.*, 2000) to test for the presence of genetically distinct clusters within our study system. We did not use any prior information about the geographic 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 (that is, the number of putatively different genetic clusters), and probability values of the data, that is $\ln\mathrm{Pr}(X/K)$, were plotted. Values of $K=1$ indicate a genetically uniform population, while values of $K=2$ and so on indicate the existence of genetically different arrays of individuals. Analyses were carried out with 100 000 iterations, following a burn-in period of 10 000 iterations. Nonetheless, testing for differences in allele frequencies between geographically distinct populations may be more useful than clustering analyses performed in STRUCTURE when genetic differentiation is weak (for example, Latch *et al.*, 2006) or affected by isolation by distance (see software documentation in http://pritch.bsd.uchicago.edu/software/structure22/readme.pdf). Thus, we employed the programme GENETIX 4.04 to calculate F_{ST} values between groups of individuals sampled from different locations of the Lesser Kestrel breeding distribution. Although the distribution range of the Eurasian Kestrel is relatively continuous, we also calculated F_{ST} values between distant sampled locations to contrast F_{ST} pair-wise values with STRUCTURE results. The significance of F_{ST} pair-wise 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). Isolation by distance was investigated through Mantel tests based on the traditional $F_{ST}/1-F_{ST}$ approach. We introduced in the programme GENETIX a matrix containing values of genetic differentiation between each pair of sampled populations (that is, $F_{ST}/1-F_{ST}$ values represented in the y axis) plus a matrix containing the geographical distance in kilometres between each pair of sampled locations (represented in the x axis). Geographical distances were calculated according to a straight line connecting the geometrical centre of each pair of sampled populations. Calculations were accomplished by using a scaled map

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and a ruler. The significance of the correlation between genetic differentiation and geographical distance was tested in GENETIX 4.04 through a *P*-value calculated using 10 000 permutations.

Allelic richness, average observed heterozygosities and the inbreeding coefficient F_{IS} among groups of samples encompassing individuals from different species or subspecies were compared using the permutation test ($N = 10 000$) implemented in FSTAT (Goudet, 2001). The allelic richness estimate, which is calculated from random permutations of a minimum shared number of individuals between groups, is especially useful in this study as highly polymorphic loci such as Fp79-4 may decisively bias estimates of genetic diversity in relation to sample size. The non-parametric Wilcoxon test was also employed to detect significant differences between sampled locations in polymorphism statistics obtained at each locus (that is, allelic richness and average observed heterozygosities). Finally, microsatellite diversity at each pair of locations, measured as the mean number of alleles per individual, was compared using Student's *t*-tests.

Results

Hardy–Weinberg equilibrium and genetic diversity

Overall, 103 alleles were found in 320 Lesser Kestrels, 75 alleles in 128 mainland Eurasian Kestrels and 46 alleles in 28 island Eurasian Kestrels (see Table 2). Locus *Fp107* departed significantly from Hardy–Weinberg expectations, showing heterozygosity deficits in most populations that are probably explained by the presence of null alleles (see also Alcaide *et al*., 2005). As null alleles may violate several assumptions of the genetic methods we intended to apply, locus *Fp107* was removed from further analysis. Mainland populations from both Kestrel species fitted to Hardy–Weinberg expectations after excluding this locus. We found, in contrast, statistically significant heterozygosity deficits, even after Bonferroni corrections

for multiple tests, in the smallest insular population corresponding to *F. t. dacotiae*.

Population differentiation

In Lesser Kestrels, the Bayesian analysis of population structure excluding any *a priori* information about the origin of individuals indicated panmixia (that is, $K = 1$, see Figure 2) as the most likely scenario. Nevertheless, traditional estimates of population differentiation relying on differences in allele frequencies revealed weak ($F_{ST} < 0.055$) but significant patterns of genetic differentiation, even after Bonferroni corrections for multiple tests, when we compared geographically distinct populations (Table 3). In fact, genetic divergence across the study area conformed significantly to an isolation-by-distance pattern (Figure 3).
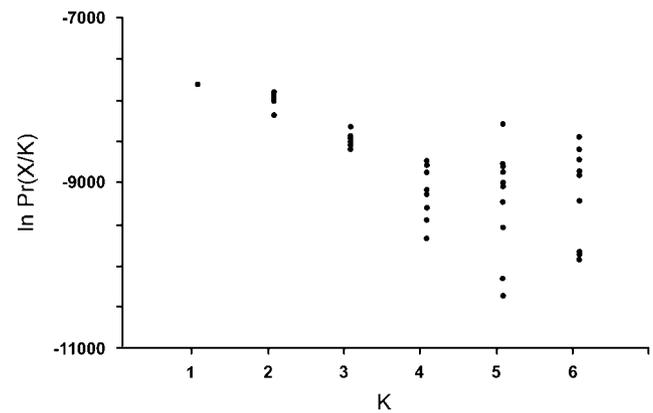

Figure 2 Bayesian clustering analysis of 320 Lesser Kestrels sampled in different regions of the Western Palearctic. For each value of *K* (that is, the number of putatively different genetic clusters tested), 10 simulations were carried out to obtain the probability of the data (*y* axis).

Table 2 Number of alleles across nine microsatellite markers in the Lesser Kestrel (*Falco naumanni*), the European subspecies of the Eurasian Kestrel (*Falco tinnunculus tinnunculus*) and the two subspecies of the Eurasian Kestrel inhabiting the Canary Islands (*Falco tinnunculus canariensis* and *Falco tinnunculus dacotiae*)

Locus range size (bp)	Falco naumanni (n = 320)	Falco t. tinnunculus (n = 128)	Falco t. canariensis (n = 12)	Falco t. dacotiae (n = 16)
Fp5	7	8	7	7
	99–111	101–115	101–113	101–113
Fp13	5	4	2	4
	86–106	92–98	92–94	92–98
Fp31	8	7	3	2
	124–142	128–142	134–138	134–138
Fp46-1	10	6	4	6
	115–139	117–127	119–125	115–125
Fp79-4	35	19	6	8
	125–192	129–154	137–149	137–152
Fp89	4	5	2	4
	116–122	116–124	118–120	116–122
Fp107	17	17	5	5
	185–231	195–233	193–221	193–221
Cl347	11	9	5	5
	96–116	100–116	100–112	100–112
Fp5	6	NA	NA	NA
	118–123	NA	NA	NA

Abbreviation: NA, not applicable.
The number of individuals analysed for each species or subspecies is shown in parentheses.

On the other hand, the clustering analysis implemented in STRUCTURE detected only two genetically distinct clusters within Eurasian Kestrels (that is, $K = 2$)

Table 3 Pair-wise F_{ST} values (above diagonal) and corresponding *P*-values (below diagonal) between Lesser Kestrel populations from the Western Paleartic

	NES	CWS	SWS	FRA	ITA	GRE	ISR
NES (68)		**0.008**	**0.008**	**0.014**	0	**0.009**	**0.035**
CWS (76)	<0.001		0.001	**0.019**	**0.016**	**0.014**	**0.041**
SWS (69)	0.0012	0.19		**0.023**	**0.013**	**0.013**	**0.038**
FRA (26)	0.0021	<0.001	<0.001		0.009	**0.041**	**0.034**
ITA (26)	0.56	<0.001	0.0048	0.0664		**0.017**	**0.021**
GRE (21)	0.002	0.0026	0.002	0.001	0.005		**0.054**
ISR (34)	<0.001	<0.001	<0.001	0.001	0.006	<0.001	

Sample sizes at each location are indicated in parentheses. Significant values after Bonferroni corrections for multiple tests are outlined in bold. Non-Bonferroni-corrected *P*-values are given below the diagonal. See Figure 1 for geographical locations.

that distinguished the mainland subspecies against the two insular subspecies. This finding agrees with the comparably high and statistically significant pair-wise F_{ST} values reported between Eurasia and the Canary Islands ($F_{ST} > 0.075$, all Bonferroni-corrected *P*-values <0.05; Table 4). Conversely, there was no evidence for genetic subdivision within Eurasia, as none of the pair-wise F_{ST} values were significantly different from zero ($F_{ST} < 0.015$, all non-Bonferroni-corrected *P*-values >0.05), or within the Canarian Archipelago ($F_{ST} = -0.018$, $P = 0.87$) (see Table 5). Contrary to Lesser Kestrels, our set of genetic markers did not reveal significant evidence of isolation by distance in the mainland subspecies of the Eurasian Kestrel (Figure 3). To compare data from both species, we performed a generalized linear model with F_{ST} as the response variable and species identity and Euclidean distance between the populations as independent variables. After conservatively adjusting the denominator degrees of freedom to compensate for the non-independence
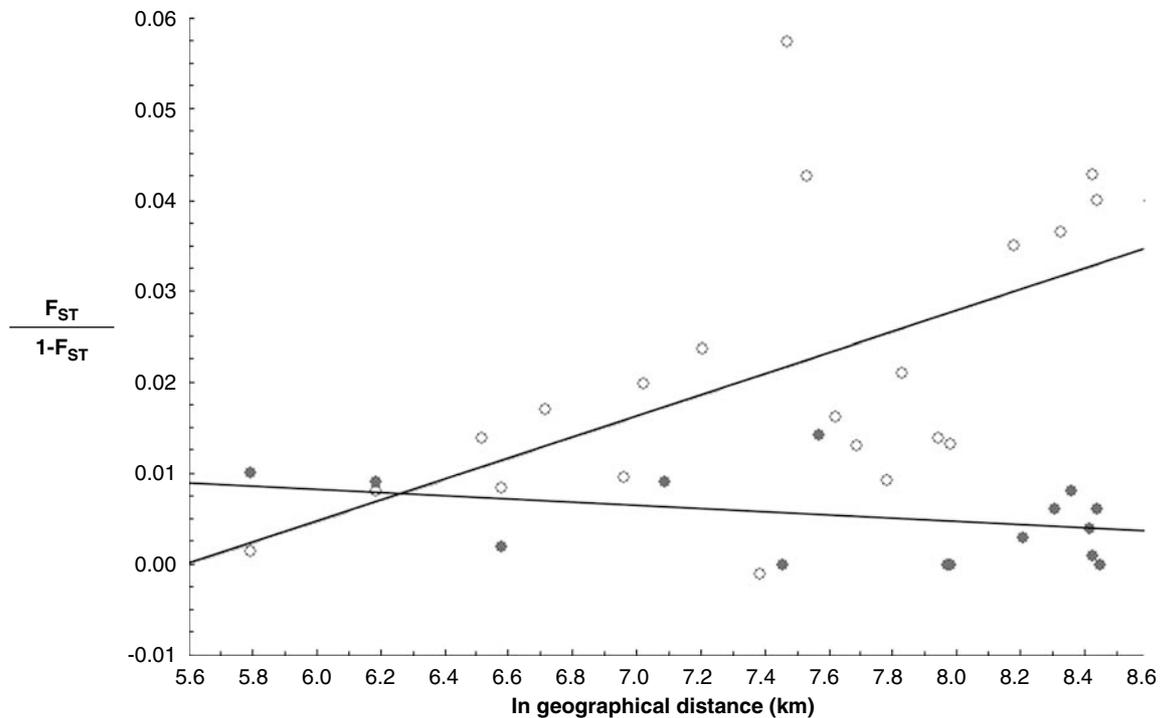

Figure 3 Relationships between the extent of genetic differentiation and geographical distance in the Lesser Kestrel (open dots, $r = 0.50$, $P = 0.04$) and Eurasian Kestrel (black dots, $r = -0.44$, $P = 0.84$) populations sampled across the Western Paleartic.

Table 4 Pair-wise F_{ST} values (above diagonal) and corresponding *P*-values (below diagonal) between Eurasian Kestrel populations from the Western Paleartic and the Canary Islands

	NES	CWS	SWS	SWI	FIN	ISR	TF	FV
NES (18)		0.009	0.002	0.009	0.006	0.008	**0.066**	**0.083**
CWS (18)	0.34		0.010	0	0.004	0	**0.103**	**0.121**
SWS (19)	0.14	0.35		0.014	0	0.006	**0.078**	**0.107**
SWI (26)	0.19	0.53	0.09		0	0.003	**0.077**	**0.099**
FIN (23)	0.23	0.29	0.49	0.60		0.001	**0.078**	**0.105**
ISR (24)	0.18	0.42	0.22	0.31	0.39		**0.077**	**0.105**
TF (12)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		-0.018
FV (16)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.87	

Sample sizes at each location are indicated in parentheses. Significant values after Bonferroni corrections for multiple tests are outlined in bold. Non-Bonferroni-corrected *P*-values are given below the diagonal. See Figure 1 for geographical locations.

Table 5 Comparison of average genetic estimates among groups of Kestrel populations that was performed using the permutation test ($N = 10\,000$) implemented in the programme FSTAT

	Allelic richness	Observed heterozygosity	Inbreeding coefficient (F_{IS})
Lesser Kestrel	5.82	0.66	0.024
Eurasian Kestrel (Mainland)	5.28	0.66	0.084
Eurasian Kestrel (Canary Islands)	4.24	0.46	0.265

Allelic richness was calculated over a minimum number of 12 individuals.

Table 6 Genetic diversity across eight microsatellite markers in six geographically distinct populations of Lesser Kestrels

	Allelic richness	Average observed heterozygosity	Inbreeding coefficient (F_{IS})
NES	6.6	0.63	0.07
CWS+SWS	7.06	0.65	0.05
FRA	6.02	0.60	0.04
ITA	6.89	0.67	−0.06
GRE	6.88	0.64	0.01
ISR	7.42	0.66	0.03

Allelic richness estimates were adjusted to a minimum sample size of 21 individuals. See Figure 1 for geographical locations.

between sampling locations (see Bailey *et al.*, 2007), the interaction term remained significant ($F_{1,9} = 9.11$, $P = 0.015$).

Genetic diversity

The permutation test performed in FSTAT did not reveal statistically significant differences in genetic diversity (allelic richness and average observed heterozygosity) or inbreeding (F_{IS}) between the Lesser Kestrel and the mainland subspecies of the Eurasian Kestrel (all two-sided *P*-values > 0.05, Table 5). In contrast, average observed heterozygosity was significantly lower in island than in the continental subspecies of the Eurasian Kestrel (0.46 vs 0.66, two-sided *P*-value = 0.009; Table 5), and the difference in allelic richness was marginally significant (4.24 vs 5.28, two-sided *P*-value = 0.08; Table 5). Furthermore, we found statistically significant evidences of increased inbreeding (F_{IS}) in the Kestrel genotypes from the Canary Islands (0.265 vs 0.084, two-sided *P*-value = 0.02; Table 5).

Finally, pair-wise analyses comparing locus by locus failed to detect statistically significant differences in genetic diversity between any of the geographically distinct populations of Lesser Kestrels investigated (non-parametric Wilcoxon test, all *P*-values > 0.05; see Table 6). Average microsatellite diversity per individual was not statistically different among populations either (*t*-tests, all *P*-values > 0.05), except for a couple of comparisons involving the smallest and the most geographically isolated population from Southern France. Such comparisons involved the less genetically diverse population (France) and two of the most genetically diverse (Italy and Israel, see Table 6) populations.

Discussion

We studied the genetic implications of habitat fragmentation by comparing the generalist, continuously distributed mainland subspecies of the Eurasian Kestrel and the steppe-specialist, patchily distributed Lesser Kestrel. Our findings indicate similar levels of genetic variation in both the species, but lower levels of genetic diversity in two island subspecies of Eurasian Kestrels. With respect to population differentiation, the Bayesian clustering method separated the mainland population of Eurasian Kestrels from their island counterparts. Likewise, F_{ST} analyses showed significant genetic differentiation between, but not within, these clusters. In Lesser Kestrels, STRUCTURE assigned all individuals to a single putative population. Nonetheless, the estimates of population differentiation that made use of the additional information on the geographic distribution of allele frequencies revealed low but significant levels of genetic differentiation following an isolation-by-distance model.

It is currently assumed that species thriving within a range of environmental conditions are more sensitive to habitat transformations, their distributional ranges becoming patchier and the risk for genetic drift within fragments increasing (for example, Ferrer and Negro, 2004). Our empirical approach exemplifies a situation whereby genetic differentiation reflects the spatial distribution of populations, which, in turn, is delimited by habitat requirements. Thus, genetic differentiation between Lesser Kestrel populations increases with geographical distance (see also Alcaide *et al.*, 2008 for data on MHC genes). Even though Lesser Kestrel is a long-distance migratory species, gene flow is restricted over short distances due to high natal and breeding philopatry (Negro *et al.*, 1997; Serrano *et al.*, 2001; Serrano and Tella, 2003). Elsewhere, we found, however, a lack of fine-scale patterns of genetic differentiation in a spatially structured population of Lesser Kestrels located in northeastern Spain (Alcaide *et al.*, in press). This finding was attributed to the fact that population subdivision at the geographical scale studied (about $10\,000\,\text{km}^2$) may not have been sufficient, given the long-distance dispersal capabilities displayed by the species; consequently, gene flow had homogenized allele frequencies. Nonetheless, effective long-distance dispersal by Lesser Kestrels ($>100\,\text{km}$) has rarely been documented by direct observations (Prugnolle *et al.*, 2003; Serrano *et al.*, 2003; P Pilard and F Martín, personal communication; D Serrano *et al.*, unpublished data; M Alberdi, personal communication), a fact that would be in agreement with the emergence of genetic structuring at large geographical scales. In contrast, it has been shown in several European populations of Eurasian Kestrels that natal dispersal regularly occurs over large distances (for example, Snow, 1968; see also Korpimäki, 1988; Village, 1990; Korpimäki *et al.*, 2006; Vasko, 2007). This high amplitude of dispersal, combined with the low incidence of habitat fragmentation in the Eurasian Kestrel, would therefore explain its genetic uniformity.

Population genetics theory predicts that reductions in population size and limited migration decrease local genetic variation, triggering negative genetic processes such as inbreeding depression and loss of adaptive potential (Frankham *et al.*, 2002). Following these

predictions, recent studies in the Lesser Kestrel have repeatedly found weak positive correlations between fitness component traits and individual genetic diversity at 11 polymorphic microsatellite markers (Ortego et al., 2007b, c). However, our genetic analyses, relying on at least six microsatellites previously amplified by Ortego and co-workers (Fp5, Fp13, Fp31, Fp46-1, Fp79-4 and Fp89), have not revealed comparably low levels of microsatellite diversity or increased inbreeding in Lesser Kestrels in relation to the putatively outbred subspecies of the Eurasian Kestrel. Genetic variation at functionally and evolutionary relevant MHC loci have also shown extraordinary levels of polymorphism (>100 alleles at a single locus) and heterozygosities above 95% in Lesser Kestrels (Alcaide et al., 2008).

We believe that additional analyses of the pre-bottlenecked population are needed to evaluate the degree of genetic depauperation in the Lesser Kestrel. In any case, it appears incautious to assume that the population decline experienced by this species is likely to have translated into reduced levels of contemporary genetic variation and increased inbreeding. For instance, Brown et al. (2007) have recently failed to detect signatures of a genetic bottleneck in peregrine falcons after a devastating decline in the mid-twentieth century due to organochlorine contaminants. Similarly, some Lesser Kestrel populations have been known to experience demographic growth, either naturally (for example, Tella et al., 1998; Ortego et al., 2007a) or by means of reintroduction or supplementation programmes (for example, Pomarol, 1993). Yet even in the bottlenecked and geographically isolated population from Southern France, from where we report the lowest levels of microsatellite polymorphism (Table 6), there is no documented evidence of a relationship between inbreeding and fitness. Conversely, local first-year survival in Southern France was similar or even higher than that in Spain (Hiraldo et al., 1996; Prugnolle et al., 2003; D Serrano, unpublished data), which suggests that ecological constraints may play a more prominent role in individual fitness than genetic diversity.

Our genetic analyses also indicate that genetic drift has provoked weak but significant fluctuations in allele frequencies ($F_{ST} < 0.05$) in Lesser Kestrels, but migration might have mitigated allele fixation (see for instance Mills and Allendorf, 1996). In fact, it has been theoretically concluded that the rule of one migrant per generation is sometimes sufficient to maintain genetic diversity while allowing some divergence between fragmented populations (reviewed by Keighobadi, 2007). Moreover, interpopulation differentiation is tough to proceed faster than loss of genetic variation after habitat fragmentation (for example, Keyghobadi et al., 2005). Long-distance dispersal events connecting adjacent populations of Lesser Kestrels have been detected. For instance, several birds ringed as nestlings in the Iberian Peninsula have been resighted as breeding birds in Southern France, covering dispersal distances of up to 1000 km (Prugnolle et al., 2003; P Pilard, personal communication). Such effective dispersal displacements provide opportunities for genetic rescue (for example, Vilà et al., 2003), probably explaining why Lesser Kestrels do not show reduced genetic diversity when compared with the continental subspecies of the Eurasian Kestrel. The comparison between continental and insular subspecies of the Eurasian Kestrel, using the same genetic methods, provides a valuable supporting reference in this respect. Restricted gene flow is expected to accelerate genetic divergence (Table 4), loss of genetic variation and increased inbreeding. These predictions are in accordance with our estimates of genetic diversity (Table 5) and also with other comparisons between mainland and insular populations of Kestrels (for example, Nichols et al., 2001).

In conclusion, this study illuminates the genetic consequences of habitat fragmentation in open populations of birds of prey. Even though habitat loss, population decline and restricted gene flow over short distances may increase genetic divergence, low rates of long-distance dispersal may counteract the loss of genetic variation through genetic drift.

Acknowledgements

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