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Individual genetic diversity correlates with the size and spatial isolation of natal colonies in a bird metapopulation

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The genetic consequences of small population size and isolation are of central concern in both population and conservation biology. Organisms with a metapopulation structure generally show effective population sizes that are much smaller than the number of mature individuals and this can reduce genetic diversity especially in small sized and isolated subpopulations. Here, we examine the association between heterozygosity and the size and spatial isolation of natal colonies in a metapopulation of lesser kestrels (*Falco naumanni*). For this purpose, we used capture–mark–recapture data to determine the patterns of immigration into the studied colonies, and 11 highly polymorphic microsatellite markers that allowed us to estimate genetic diversity of locally born individuals. We found that individuals born in smaller and more isolated colonies were genetically less diverse. These colonies received a lower number of immigrants, supporting the idea that both reduced gene flow and small population size are responsible for the genetic pattern observed. Our results are particularly intriguing because the lesser kestrel is a vagile and migratory species with great movement capacity and dispersal potential. Overall, this study provides evidence of the association between individual heterozygosity and the size and spatial isolation of natal colonies in a highly mobile vertebrate showing relatively frequent dispersal and low genetic differentiation among local subpopulations.

Keywords: dispersal; *Falco naumanni*; genetic diversity; immigration; population isolation; population size

1. INTRODUCTION

Numerous organisms are distributed forming spatially structured populations in the manner of metapopulations, composed of a number of local populations differing in size and degree of connection that are generally submitted to extinction–colonization dynamics and behave as highly heterogeneous systems (Levins 1969; Hanski 1998). From a genetic point of view, organisms with a metapopulation structure generally show effective population sizes that are much smaller than the number of mature individuals (Gilpin 1991; Hedrick 1996; Amos & Harwood 1998). This has special relevance for small and isolated subpopulations, because they are theoretically more likely to exhibit reduced genetic diversity and may be more prone to extinction from genetic and stochastic processes than larger and better connected ones (Frankham 1995, 2005; Saccheri *et al.* 1998; Nieminen *et al.* 2001; Spielman *et al.* 2004).

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Matings between closely related parents reduce genetic diversity and this frequently results in progeny with lower fitness than outbred ones (Charlesworth & Charlesworth 1987; Falconer & Mackay 1996). In natural populations, many empirical studies have also provided supporting evidence for a positive relationship between individual genetic diversity measured at neutral markers and different components of fitness, including disease resistance (Acevedo-Whitehouse *et al.* 2003, 2006; Ortego *et al.* 2007*a*), fecundity (Ortego *et al.* 2007*b*) and survival probability (Hoffman *et al.* 2004; Markert *et al.* 2004), although the possible role of inbreeding in such correlations has been recently put into question (Balloux *et al.* 2004; Pemberton 2004). At the population level, low genetic diversity is suspected to reduce the ability of populations to respond to novel and changing environmental conditions (Willi *et al.* 2006) and compromise their long-term viability (Saccheri *et al.* 1998; Westemeier *et al.* 1998; Nieminen *et al.* 2001; Spielman *et al.* 2004; Frankham 2005). The level of genetic variation within a population depends on a balance between mutation, natural selection, genetic drift, inbreeding and gene flow, the last four factors being closely linked to the size and spatial isolation of populations (Frankham 1996; Hedrick 2000). Thus, it is not very surprising that the study of the genetic and demographic consequences of small

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population size and isolation is of central concern in both population and conservation biology (Frankham 1996; Amos & Balmford 2001).

Empirical evidence of the genetic consequences of small population size has been established in comparisons across species, with a number of studies reporting a positive relationship between genetic diversity and estimated effective population sizes (e.g. Soulè 1976; Nevo *et al.* 1984; but see Bazin *et al.* 2006). Within species, most studies have predominantly focused on analysing differences in genetic diversity among isolated populations that generally follow an 'island' model of spatial genetic structure due to the presence of natural or anthropogenic dispersal barriers that prevent gene flow among populations. These studies generally include analyses of islands (e.g. Frankham 1997; Whiteman *et al.* 2006; White & Searle 2007) or isolated populations (e.g. Dixon *et al.* 2007) differing in size and have generally observed reduced levels of genetic diversity in smaller populations. Finally, other studies have found higher variability in continuous populations compared with isolated populations (e.g. Segelbacher *et al.* 2003; Hoglund *et al.* 2007; White & Searle 2007), or reported temporal increases of genetic diversity following demographic expansions (e.g. Hansson *et al.* 2000; Ortego *et al.* 2007*c*), or decreases after population bottlenecks (e.g. Groombridge *et al.* 2000; Taylor *et al.* 2007). The association between genetic diversity and the degree of connection/size of local subpopulations has also been extensively studied in a metapopulation context (e.g. Hanfling & Brandl 1998; Saccheri *et al.* 1998; Rowe *et al.* 1999; Harper *et al.* 2003; Andersen *et al.* 2004), although empirical evidence for such an association in highly mobile species showing frequent dispersal and scarce genetic differentiation among local subpopulations is much more scarce (e.g. Seppa & Laurila 1999).

An ideal vertebrate model to study the association between genetic diversity and the size and spatial isolation of local populations within a classical metapopulation system is the lesser kestrel (*Falco naumanni*). The lesser kestrel is a small size bird of prey that forms breeding colonies experiencing relatively frequent colonization and extinction events that are highly dependent on a balance between adult survival, breeding performance and migration processes (Serrano *et al.* 2004; Aparicio *et al.* 2007; Calabuig *et al.* 2008). The aim of this study was to analyse the association between individual genetic diversity and the size and spatial isolation of natal colonies in a metapopulation of lesser kestrels. We first studied variability among colonies in immigration patterns in relation to their size and spatial isolation and then we analysed whether heterozygosity of locally born individuals was associated with these parameters. For this purpose, we used capture–mark–recapture data to determine the patterns of immigration into the studied colonies, and 11 highly polymorphic microsatellite markers that allowed us to estimate genetic diversity of locally born individuals.

2. MATERIAL AND METHODS

(a) *Study population and field procedures*

The study was conducted in La Mancha, central Spain, in an area covering approximately 1000 km^2 (figure 1). In this area, lesser kestrels form colonies in abandoned farm houses where they nest under tiled roofs and inside holes in walls. Each building or complex of buildings occupied by at least two pairs was defined as a breeding colony. During the years 2000–2006, we have studied a total of 37 breeding colonies clustered in two subpopulations separated by 30 km: 'Villacañas' (39°30′ N, 3°20′ W; 17 colonies) and 'Consuegra' (39°35′ N, 3°40′ W; 6 colonies) subpopulations (figure 1). However, in spite of the low exchange of individuals between both subpopulations, Bayesian model-based clustering analyses (STRUCTURE v. 2.1, Pritchard *et al.* 2000) indicated that they are not genetically differentiated (maximum number of clusters modelled = 10; Ortego *et al.* 2007*a*, 2008).

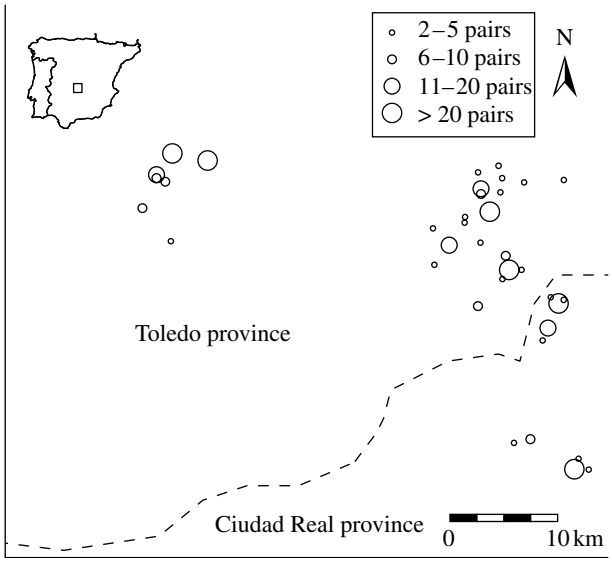

Figure 1. Map of the study area showing the spatial distribution and average size (number of breeding pairs) of the studied lesser kestrel colonies.

Monitoring included the capture and banding of breeding adults, recording of breeding parameters and intensive ringing of nestlings in the colonies (Ortego *et al.* 2007*a–c*). During the study period (2000–2006 breeding seasons), we ringed almost all nestlings born in the study area (approx. 95% of nestlings; approx. 400 chicks per year) and the effectiveness of capture (i.e. the ratio between the number of captured birds and the total number of individuals at a colony) of breeding adults in the studied colonies was on average 70% (Ortego *et al.* 2007*c*, 2008). Adults were trapped with a noose carpet or by hand during incubation and were individually marked with metallic and coloured plastic rings for later identification. Chicks were marked at hatching with a waterproof felt-tip pen, and were banded 5–7 days later. Blood samples (100 µl) for genetic analyses were obtained by venipuncture of the brachial vein and preserved in approximately 1200 µl ethanol 96% at −20°C. All ringed chicks were bled when they were 30 days old.

(b) *Characteristics of the colonies*

Colonies were characterized between 2000 and 2006 in terms of size (number of breeding pairs) and isolation (distance and sizes of neighbouring colonies). From 30 April onwards, each hole apparently appropriate for lesser kestrels was regularly examined to determine the total number of occupied nests and calculate the total number of breeding pairs in the colonies (Aparicio *et al.* 2007). We estimated the spatial

isolation of the colonies using different measurements: (i) distance to the nearest colony, calculated as the straight-line distance to the nearest neighbour colony of lesser kestrels and (ii) population connectivity (Hanski 1998). Connectivity (S) of colony i is calculated as: $S_{i,t} = \sum_{j \neq i} \exp(-\alpha d_{ij}) N_{j,t}$, where $N_{j,t}$ is number of breeding pairs in colony i; d_{ij} is the distance between colonies i and j; and $1/\alpha$ is the average dispersal distance, set to 3.022 km based on previous research in the study population (J. Ortego 2007, unpublished manuscript). It should be noted that isolated colonies are not the smallest ones in our study population (Spearman rank correlation coefficients; colony size–distance to the nearest colony: $r_s = 0.162$, $p = 0.460$; colony size–connectivity: $r_s = 0.061$, $p = 0.783$).

(c) *Immigration patterns*

To study immigration patterns, we determined the number of non-local birds arriving every year to a given colony. We calculated immigration rates using only recovery data from banded individuals, excluding from the analyses information on unringed birds of uncertain origin. We considered immigrants for a given colony those banded birds that were born or bred in other colonies in previous seasons (i.e. individuals with exact known origin). Thus, we are confident that our dataset is not biased due to unmarked individuals that may be philopatric but considered as immigrants merely because we failed to capture them in previous breeding seasons.

(d) *Genotyping and genetic diversity estimates*

We genotyped 419 nestling lesser kestrels across 11 highly polymorphic microsatellite markers: 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.* 2007*d*; see Ortego *et al.* 2007*a* for microsatellite details). All individuals were genotyped at all these 11 microsatellite markers. We used QIAamp DNA Blood Mini Kits (QIAGEN) to extract and purify genomic DNA from the blood samples. Approximately 5 ng of template DNA was amplified in 10 µl reaction volumes containing 1× reaction buffer (67 mM Tris–HCl (pH 8.3), 16 mM $(NH_4)_2SO_4$, 0.01% Tween-20, EcoStart Reaction Buffer, Ecogen), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 µM of each dye-labelled primer (FAM, HEX or NED) and 0.1 U of *Taq* DNA EcoStart polymerase (Ecogen). All reactions were carried out on a Mastercycler EpgradientS (Eppendorf) thermal cycler. The PCR programme used was 9 min denaturing at 95°C followed by 30 cycles of 30 s at 94°C, 45 s at the annealing temperature (Ortego *et al.* 2007*d*) and 45 s at 72°C, ending with a 5 min final elongation stage at 72°C. Amplification products were electrophoresed using an ABI 310 Genetic Analyzer (Applied Biosystems) and genotypes were scored using GeneScan v. 3.7 (Applied Biosystems). We used two metrics to estimate individual genetic diversity: (i) uncorrected heterozygosity (H_O), calculated as the proportion of loci at which an individual is heterozygous and (ii) homozygosity by locus (HL), a microsatellite derived measure that improves heterozygosity estimates in open populations by weighting the contribution of each locus to the homozygosity value depending on its allelic variability (Aparicio *et al.* 2006; Ortego *et al.* 2007*b*). Particularly, HL improves heterozygosity estimates when markers are highly different in variability, as is the case in this study (Ortego *et al.* 2007*a*; e.g. Ortego *et al.* 2007*c*). HL is calculated as follows: $HL = (\Sigma E_h)/(\Sigma E_h + \Sigma E_j)$, where E_h and E_j are the expected heterozygosities of the loci that an individual bears in homozygosis (h) and heterozygosis (j), respectively (Aparicio *et al.* 2006). H_O and HL were calculated using Cernicalin, an excel spreadsheet available on request.

(e) *Statistical analyses*

(i) *Patterns of immigration*

We examined the factors influencing the number of immigrants arriving to a given colony using generalized linear mixed models (GLMMs) implemented with the GLIMIX macro of SAS (SAS Institute 2004). GLMMs allow analyses of data where the response variable is determined by both random and fixed effects. Total number of immigrants (88 colony-years; range: 5–20 colonies per year) was analysed using a Poisson error structure and log link, including as covariates colony size, distance to the nearest lesser kestrel colony (transformed as $\log(x+1)$), and population connectivity (S). We also included a variable that we defined as effectiveness of capture calculated as the ratio between the number of captured birds and the total number of individuals at a colony in a given year. We did so because the number of observed immigrants in the colonies is likely to be affected by that parameter. As several colonies were monitored across years, we included year and colony identity as random effects in all these analyses in the manner of a randomized complete block design to avoid pseudo-replication (Krackow & Tkadlec 2001). Subpopulation identity was also included as random effect to control for the potential non-independence of number of immigrants within subpopulations.

(ii) *Patterns of genetic diversity*

We used GLMMs to study the factors determining genetic diversity of locally born individuals in relation to colony size, distance to the nearest lesser kestrel colony (transformed as $\log(x+1)$) and population connectivity (S). H_O and HL of locally born individuals (419 individuals from 196 nests) were fitted as dependent variables using a normal error structure and identity link. The identities of colonies, cohorts and subpopulations were included as random effects to control for the potential non-independence of H_O and HL within colonies, cohorts and subpopulations. Given that siblings were not independent among them, we also included brood identity nested within colony identity (i.e. a higher level factor; Singer 1998) as random effect.

Finally, we also used a GLMM to analyse the factors determining genetic diversity at the population level (i.e. colonies) rather than at the individual level. For this purpose, we calculated average heterozygosity for each colony and year. We did not pool data over years for each colony because both colony size and parameters associated with spatial isolation are not constant over years for a given colony as a consequence of population changes and eventual extinction or foundation of new colonies (Ortego *et al.* 2007*c*). We performed these analyses taking into account that data of genetic diversity provided by siblings are non-independent. Thus, to avoid pseudo-replication, we dealt with the means of heterozygosity for each brood and then calculated average heterozygosity for each colony-year (e.g. Ortego *et al.* 2007*c*). The identities of colonies, cohorts and subpopulations were also included as random effects in this analysis.

Initially, each GLMM was constructed with all explanatory terms fitted, including first-order interactions and quadratic effects to account for potential nonlinear

Table 1. GLMM (Poisson error and log link function) for total number of immigrants arriving to a focal colony. (Distance to the nearest colony was transformed as $\log(x+1)$.)

	estimate ± s.e.	test	p
intercept	−1.261 ± 0.477		
squared distance to the nearest colony (m)	−3.248 ± 1.192	$F_{1,84}=7.42$	0.008
colony size	0.052 ± 0.011	$F_{1,84}=23.26$	<0.001
effectiveness of capture	1.343 ± 0.464	$F_{1,84}=8.40$	0.005
colony identity	0.271 ± 0.184	$Z=1.47$	0.071
subpopulation	0	—	—
year	0.525 ± 0.473	$Z=1.11$	0.134

relationships. Final models were selected following a backward procedure, by progressively eliminating non-significant variables. The significance of the remaining variables was tested again until no additional variable reached significance. The result is the minimal most adequate model for explaining the variability in the response variable, where only the significant explanatory variables are retained. All tests were performed using the residual degrees of freedom (SAS Institute 2004). Hypotheses were tested using F-statistics and all p values refer to two-tailed tests.

3. RESULTS

(a) *Patterns of immigration*

The total number of immigrants arriving at a given colony was positively associated with colony size and the effectiveness of capture, and negatively associated with squared distance to the nearest colony of lesser kestrels (table 1, figure 2). This pattern indicates that number of immigrants decreases nonlinearly with distance to the nearest colony. The total number of immigrants was not significantly associated with population connectivity ($F_{1,83}=1.40$, $p=0.240$). However, note that the distance to the nearest colony and population connectivity were intercorrelated (Spearman rank correlation coefficient; $r_s=0.486$, $p=0.019$). To address this problem of collinearity, we performed a complementary analysis to assess the effect of population connectivity, when it alone is included into the model. After excluding squared distance to the nearest colony from the model, population connectivity became positive and significantly associated with number of immigrants ($F_{1,84}=5.19$, $p=0.025$). Thus, although both variables significantly predicted the total number of immigrants arriving to a given colony, the effect of population connectivity was lower and it disappeared when squared distance to the nearest colony was included in this model. Other quadratic terms and interactions between independent variables were not significant in any analysis ($p>0.1$ in all cases).

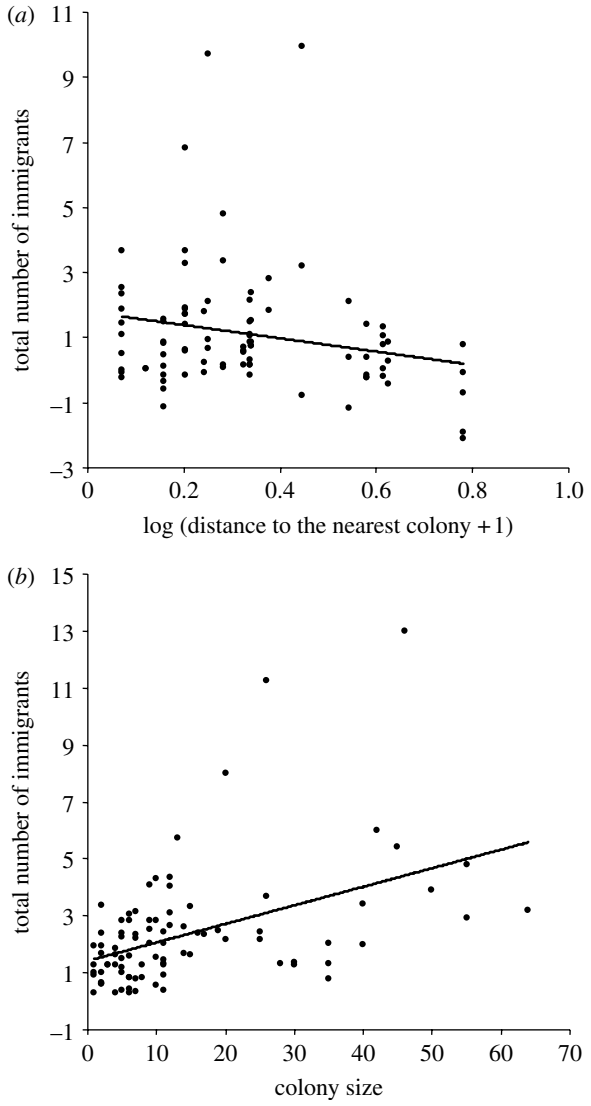

Figure 2. Relationship between (*a*) distance to the nearest colony (transformed as $\log(x+1)$) and (*b*) colony size and total number of immigrants. Total number of immigrants is expressed as statistical residuals obtained after controlling for other influencing variables.

(b) *Patterns of genetic diversity*

The measures HL and H_O were highly correlated (Pearson correlation; $r=-0.974$, $p<0.001$). Under a wide range of simulated scenarios, HL has been proved to be a better predictor of genome-wide heterozygosity than H_O in open populations (Aparicio *et al.* 2006). Furthermore, HL generally provides a better fit of the data than other measures of multilocus heterozygosity in both this and previous genetic studies of our lesser kestrel population (Ortego *et al.* 2007*a–c*). For these reasons, detailed model parameters and graphical outputs are only presented for HL analyses in tables 2 and 3 and figures 3 and 4, respectively, and the same information for H_O is given in the electronic supplementary material. After controlling for random effects, the genetic diversity of locally born individuals (nestlings) was negatively associated with squared distance to the nearest colony of lesser kestrels (table 2, figure 3*a*) and positively associated with colony size (table 2, figure 3*b*). Once again, the effect of population connectivity disappeared (H_O: $F_{1,415}=1.13$, $p=0.288$; HL: $F_{1,415}=0.86$, $p=0.355$) when squared

Table 2. GLMM (normal error and identity link function) for HL of locally born individuals. (Distance to the nearest colony was transformed as $\log(x+1)$.)

	estimate ± s.e.	test	p
intercept	0.327 ± 0.014		
squared distance to the nearest colony (m)	0.153 ± 0.056	$F_{1,416}=7.34$	0.007
natal colony size	−0.002 ± 0.001	$F_{1,416}=6.34$	0.012
brood identity	0.0017 ± 0.0009	$Z=1.84$	0.033
colony identity	0.0003 ± 0.0005	$Z=0.66$	0.253
subpopulation	0	—	—
cohort	0	—	—

Table 3. GLMM (normal error and identity link function) for average colony HL. (Distance to the nearest colony was transformed as $\log(x+1)$.)

	estimate ± s.e.	test	p
intercept	0.316 ± 0.016		
squared distance to the nearest colony (m)	0.234 ± 0.065	$F_{1,51}=12.77$	<0.001
colony size	−0.002 ± 0.001	$F_{1,51}=6.96$	0.011
colony identity	0.0007 ± 0.0009	$Z=0.74$	0.230
subpopulation	0	—	—
cohort	0	—	—

distance to the nearest colony was retained in the model. Other quadratic terms and interactions between independent variables were not significant in any of these analyses ($p>0.1$ in all cases).

Finally, average colony genetic diversity was negatively associated with squared distance to the nearest colony of lesser kestrels (table 3, figure 4*a*) and positively associated with colony size (table 3, figure 4*b*). Other quadratic terms and interactions between independent variables were not significant in these analyses ($p>0.1$). The precision of average colony heterozygosity could be variable because sample size (i.e. number of genotyped broods) varied between colonies. To avoid this problem, we performed a complementary analysis to reinforce the results reported above. Given that random terms were not significant in the multivariate mixed model (table 3), we used sample size to give observations of different weights in a weighted least-squares analysis (e.g. Kaeuffer *et al.* 2007; Ortego *et al.* 2007*c*). As above, average colony heterozygosity was negatively associated with squared distance to the nearest colony of the lesser kestrels (H_O: $t=-3.521$, $p=0.001$; HL: $t=3.190$, $p=0.002$) and positively associated with colony size (H_O: $t=3.521$, $p=0.001$; HL: $t=-2.753$, $p=0.008$).

(c) *Patterns of immigration/genetic diversity and geographical location of colonies*

Empirical and theoretical studies have established that the geographical location of populations is a major determinant of several population characteristics such as their size, connectivity and genetic diversity (e.g. García-Ramos & Kirkpatrick 1997; Kirkpatrick & Barton 1997; Lammi *et al.* 1999; Vucetich & Waite 2003). To address this question and resolve the potential confounding effect of the geographical location of the studied colonies on the results reported above, we analysed whether the studied parameters (i.e. spatial isolation and size of the colonies, immigration rate, and individual and average colony heterozygosity) are associated with the geographical location of the colonies within each studied subpopulation. After controlling for other influential variables and random effects (see above), we found no association between distance from the colony to subpopulation centre and colony size ($F_{1,86}=0.23$, $p=0.632$), distance to the nearest colony ($F_{1,86}=1.95$, $p=0.166$), connectivity ($F_{1,86}=2.25$, $p=0.137$), immigration rate ($F_{1,83}=0.39$, $p=0.536$), individual heterozygosity (H_O: $F_{1,415}=1.30$, $p=0.254$; HL: $F_{1,415}=0.90$, $p=0.343$) or average colony heterozygosity (H_O: $F_{1,50}=0.53$, $p=0.472$; HL: $F_{1,50}=0.04$, $p=0.849$). Note that the absence of association between the number of immigrants and distance to subpopulation centre also indicates that immigration rates in peripherally located colonies are not substantially underestimated due to the arrival of several unringed birds from other subpopulations outside the study area.

4. DISCUSSION

Here, we show that lesser kestrels born in smaller and spatially isolated colonies are genetically less diverse than those born in larger and better connected ones. These colonies received a lower number of immigrants, supporting the idea that both reduced gene flow and small population size are responsible for the observed patterns of heterozygosity. Both the number of immigrants and heterozygosity were better explained by a nonlinear relationship with distance to the nearest colony than by the linear term, indicating that the association of this measure of spatial isolation with these parameters follows a saturation curve. This nonlinear pattern indicates that the negative association between the degree of spatial isolation and genetic diversity become relevant from a minimum distance, suggesting that the arrival of immigrants, and thus gene flow, only decreases from a threshold distance. Although the maximum distance between neighbouring colonies observed in the study population is very short in relation to the high movement capacity of lesser kestrels, the lower number of immigrants arriving at spatially isolated colonies may be associated with a lower chance of such colonies being explored by dispersing

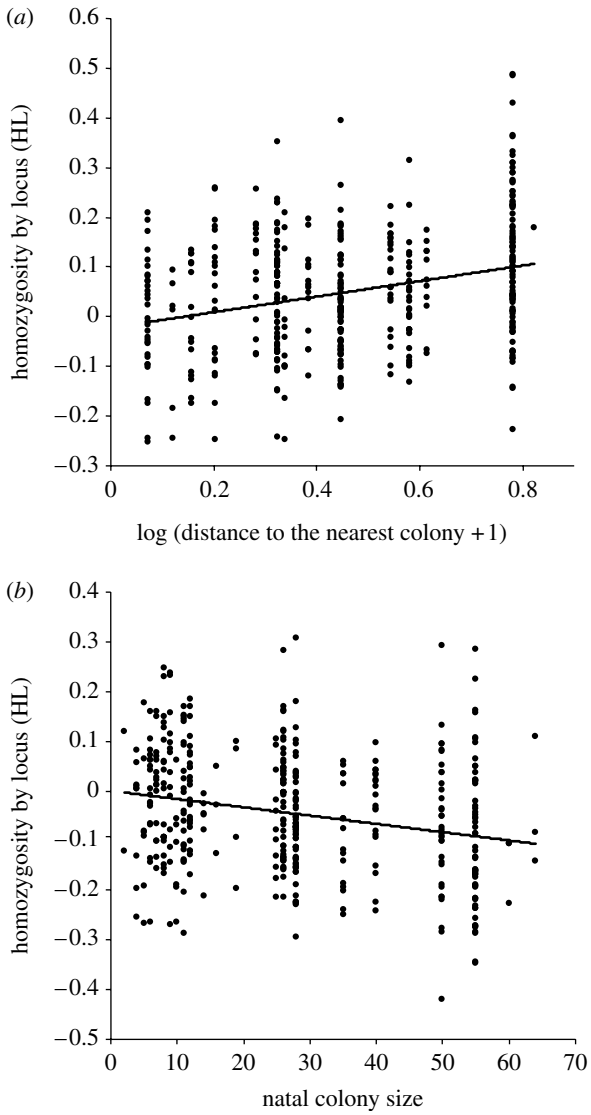

Figure 3. Relationship between (*a*) distance to the nearest colony (transformed as $\log(x+1)$) and (*b*) natal colony size and HL of locally born individuals. HL is expressed as statistical residuals obtained after controlling for other influencing variables.

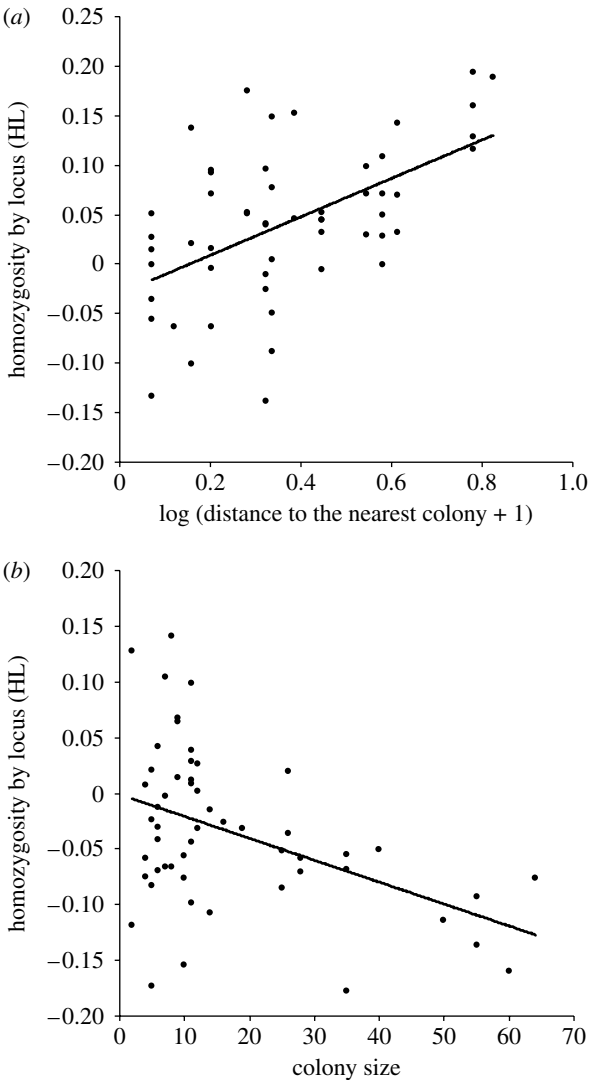

Figure 4. Relationship between (*a*) distance to the nearest colony (transformed as $\log(x+1)$) and (*b*) colony size and average colony HL. Average colony HL is expressed as statistical residuals obtained after controlling for other influencing variables.

individuals. It has been confirmed that adult lesser kestrels explore several colonies at the end of fledgling supposedly to obtain information on conspecific breeding performance, a time-consuming behaviour that is likely to reduce the chance of prospecting spatially isolated colonies due to the short time period available before all the chicks have fledged (Aparicio *et al.* 2007). On the other hand, lesser kestrels may be reluctant to disperse and settle in isolated colonies due to the benefits derived from increased local familiarity. The strong association between number of immigrants and colony size could be a consequence of the higher chance that prospecting birds detect larger colonies (Bowler & Benton 2005). Also, the higher availability of breeding cavities in larger colonies, generally located in bigger and structurally more complex buildings, could also explain why the number of immigrants arriving at a focal colony increases with its size (G. Calabuig 2007, unpublished data).

The observed pattern of heterozygosity is particularly intriguing because the lesser kestrel is a vagile and migratory species with great dispersal potential, showing dispersal distances and daily home ranges (lesser kestrels forage up to 10 km from their colony; Bonal & Aparicio 2001) much larger than the average minimum distance between two neighbour breeding colonies in our study population. Furthermore, we have studied a single metapopulation in a restricted geographical area, where lesser kestrels show a weak isolation-by-distance pattern of fine spatial scale genetic structure typical of continuous populations (Ortego *et al.* 2008; see Wright 1943; Slatkin 1993). Apart from asymmetrical migration, some life-history characteristics of the study species may have also favoured the observed pattern of individual genetic diversity. First, the general philopatric behaviour characterizing lesser kestrels (Negro *et al.* 1997; Serrano *et al.* 2001) is likely to have contributed to the observed pattern by increasing the chance of crosses between genetically similar individuals particularly in small size colonies (Ortego *et al.* 2008). Second, previous studies have found that philopatric behaviours, both in experienced and first breeders, are more prevalent in spatially isolated colonies (Serrano *et al.* 2001, 2004), which could increase the chance of crosses between relatives and favour,

together with reduced gene flow, a diminished individual genetic diversity in isolated colonies. Finally, the relatively short generation time of lesser kestrels (modal lifespan is 4 years) in comparison with other long-live species (e.g. Swart *et al.* 1994; Hailer *et al.* 2006) could also have contributed to a more frequent turnover of individuals in the breeding colonies, allowing relatively quick changes of heterozygosity at fine temporal and spatial scales (Ortego *et al.* 2007*c*, 2008).

In contrast to previous studies analysing the entire species range distribution (e.g. Lammi *et al.* 1999; Hutchison 2003), we found no difference in colony characteristics or genetic diversity between centrally and peripherally located colonies. This may be caused by the fact that the studied subpopulations are located in the core of the species distribution in the Iberian Peninsula (Ortego *et al.* 2007*c*) and does not constitute isolated metapopulations but, rather, subpopulations within the range of dispersal from other neighbouring populations (Ortego *et al.* 2007*c*). Thus, although the study area includes two well geographically determined clusters, several other populations are located close to the study area and interchange of individuals is likely to be a frequent process (Serrano & Tella 2003; Ortego *et al.* 2007*c*).

In conclusion, this study indicates that genetic diversity can be associated with the size and spatial isolation of local populations in highly mobile vertebrates with relatively frequent dispersal and scarce genetic differentiation among subpopulations. These results, together with the detrimental consequences of low heterozygosity reported in several species including that studied here (Ortego *et al.* 2007*a*,*b*), may help to explain the poorer reproductive performance and reduced long-term persistence of the small size and isolated populations (Saccheri *et al.* 1998; Madsen *et al.* 1999; Spielman *et al.* 2004; Frankham 2005).

This study conforms to the terms of the general ethical guidelines for animal welfare and nature conservation. We manipulated and banded lesser kestrels under licence from the Spanish institutional authorities (Environmental Agency of Junta de Comunidades de Castilla-La Mancha and the Ringing Office of the Ministry of Environment).

Primer sequences for microsatellite Fu1 and Fu2 were kindly provided by Jon H. Wetton (Forensic Science Service, UK). This work received financial support from the projects: CGL2005-05611-C02-02/BOS (Ministerio de Educación Ciencia) and PAI05-053 (Junta de Comunidades de Castilla-La Mancha). During this work, J.O. and G.C. were supported by predoctoral fellowships from the Junta de Comunidades de Castilla-La Mancha and the European Social Fund. We performed all the laboratory work at the Laboratory of Genetics of the IREC and fragment genotyping was performed by the Centro de Investigaciones Biológicas (CSIC) of Madrid.

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