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Linking habitat quality with genetic diversity: a lesson from great bustards in Spain

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Abstract The effects of habitat loss and fragmentation on the genetic structure and variability of wild populations have received wide empirical support and theoretical formalization. By contrast, the effects of habitat quality seem largely underinvestigated, partly due to technical difficulties in properly assessing habitat quality. In this study, we combine geographic information system (GIS)-based habitat-quality modelling with a landscape genetics approach based on mitochondrial DNA markers to evaluate the possible influence of habitat quality on the levels and distribution of genetic diversity in a range of natural populations ($n=15$) of *Otis tarda* throughout Spain. Ninety-three percent of the population represented by our countrywide sample lives in good-quality habitats, while 4.5% and 2.5% occur respectively in intermediate and poor habitats. Habitat quality was highly correlated with patch size, population size and population density, indicating the reliability and predictive power of the habitat suitability model. Genetic diversity was significantly correlated with habitat quality, size and density of the population, but not with patch size. Three of a total of 20 existing matrilineages from the species' current genetic pool are restricted to poor-quality habitats. This study therefore highlights the importance of considering both population genetics and habitat quality in a species of high conservation priority.

Keywords *Otis tarda* · Geographic information systems · Habitat suitability index · mtDNA

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Introduction

The great bustard, *Otis tarda*, is one of the most characteristic avian species of the lowland dry grassland ecosystems in Europe. Currently, the species is threatened and listed as vulnerable A2c+3c+4c version 3.1 under the current International Union for Conservation of Nature (IUCN) Red List of Threatened Species due to their severely fragmented habitat and continuing decline in extent and quality of habitat (www.iucnredlist.org). A study based on nuclear and mitochondrial (mt)DNA analyses showed that in Europe great bustard populations are subdivided into two different evolutionary significant units (ESUs; Moritz 1994) respectively in the European mainland (including Ukraine and south Russia) and Spain (Pitra et al. 2000). The Iberian peninsula is home to ~27,500–30,000 great bustards, approximately 60% of the world population of this species (Palacín and Alonso 2008). For great

bustards in Spain, metapopulation structure has recently been shown based on ecological surveys (Alonso and Alonso 1996; Alonso et al. 2003, 2004) and genetic analyses (Martín et al. 2002; Alonso et al. 2009). The birds occupy patches of open, steppe-like landscapes with cereal–fallow rotations, a habitat that is particularly under threat because of agriculture intensification and land abandonment linked to the European agricultural policy. Other threats are urbanization, infrastructure expansion and, locally, reforestation. Parallel to an ongoing population decline during the 20th century throughout Europe, the Spanish population of the great bustard has fragmented in several breeding areas distributed across the regions of Madrid, Castilla-La Mancha, Extremadura, Castilla y León, Navarra, Aragón and Andalusia (Alonso et al. 2003). Populations at the periphery (the last three mentioned previously) were the most genetically differentiated from the main ones in central Spain (Alonso et al. 2009). Accordingly, peripheral populations are also found to be more susceptible to decline and extinction than populations at the core of their range (Lane and Alonso 2001; Alonso et al. 2005). These are reasons why the great bustard population in Spain, although now thought to be stable, is apparently concentrating at high-quality areas, disappearing from poor-quality ones (Alonso et al. 2004; see also Pinto et al. 2005 for the Portuguese population).

In this context, it becomes particularly important to quantify how variation in ecological factors linked to habitat quality might affect and shape observed levels of genetic variability in wild populations. The effects of habitat loss and fragmentation on the genetic structure and variability of wild populations have received wide empirical support and theoretical formalization (Frankham 1995). By contrast, the effects of habitat quality (or suitability) seem largely underinvestigated, partly due to technical difficulties in properly assessing habitat quality (Vergeer et al. 2003). There are a large variety of methods in the literature to measure it (see Johnson 2007 for a review), either assessing directly habitat attributes or recording variables from individuals/populations. Recent developments in geographic information systems (GIS) and habitat modelling provide an ideal methodological framework in which high-resolution analyses of habitat attributes can be performed at the landscape level. Models allow gaining insights into the environmental variables (such as biotic, physical, climatic or human-induced habitat conditions) acting on species survival by comparing current species distribution with the optimal values for each variable (Guisan and Zimmermann 2000; Osborne et al. 2001; Suárez-Seoane et al. 2002). The output of the models is a habitat suitability index (HSI) which ranges from 0 (low quality) to 1 (high quality). It has been widely used since the early 1980s to determine

wildlife habitat quality (U.S. Fish and Wildlife Service 1980, 1981), as García et al. (2007), among other authors, did for a fragmented population of little bustard in NW Spain. This study examines the interactions between habitat quality and genetic diversity in a range of natural populations ($n=15$) of *O. tarda* throughout the Spain and considers the implications of the results for the species' conservation. Specifically, we used a GIS-based distribution model previously developed for *O. tarda* in Spain to infer a spatially explicit HSI capable of identifying, categorizing and ranking landscape quality (Suárez-Seoane et al. 2002). The spatial pattern of the species' genetic diversity was then overlaid to address the following objectives: (1) to investigate at a nationwide scale the spatial patterns of genetic variation, (2) to evaluate the possible influence of habitat quality and some demographic variables (patch size, population size, population density) on genetic variation and (3) to identify those haplotypes and determine the proportion of the overall genetic variation of the species in the study area currently living under poor habitat conditions and deserving high conservation priority.

Material and methods

Bird data and sample collection

Data about numbers and distribution of the species were compiled from censuses carried out in Spain by different teams of observers between mid March and early April (1994–2008), when great bustards gather at their mating areas and thus are easier to count (details and references in the study of Alonso et al. 2003; Palacín and Alonso 2008). A team consisted of two observers with experience in counting bustards and knowledge of the area to be surveyed. They drove four-wheel-drive vehicles slowly through most tracks, trying to count all birds and to avoid duplicated counts. Each census was carried out from dawn to dusk, with a midday pause, when birds often sit down and are harder to detect. Sampling regions were chosen to represent the distribution range of the species in Spain (Alonso et al. 2003). The corresponding population and patch sizes were delimited according to discontinuities in the distribution of both, birds and areas of steppe-like habitat, considering the movements of a large sample of radio-tagged individuals (more than 700 birds in total, 1991–2009, unpublished data). A total of 302 tissue samples of blood, feathers or unfertilised eggs from various Spanish regions were collected from breeding adults or chicks throughout the great bustard breeding range in Spain. The UTM coordinates were determined for each sample using a GARMIN-12 GPS.

Genetic analysis

DNA extraction, polymerase chain reaction (PCR) amplification of a 657 base pair (bp) fragment of the mitochondrial control region (Domain I=283 bp, Domain II=374 bp), and DNA sequencing were performed using the protocols outlined by Martín et al. (2002). The PCR products were purified (QIAquick PCR purification kit/Qiagen), sequenced by the extension-dideoxy-chain termination method (Sanger et al. 1977) with a commercial kit (Dye Terminator Cycle Sequencing Kit, Applied Biosystems/Perkin Elmer) and analysed on a 373 A sequencer (Applied Biosystems). Sequences were aligned manually and all variable sites were confirmed by visual inspection of the chromatograms. Sequences of the same haplotype were identified using the computer program Collapse 1.1 (Posada and Crandall 1998). The resulting 20 different haplotypes were submitted to the GenBank database and included in the subsequent analysis (Table 1). Initial sequence comparisons and measures of genetic variability (haplotype diversity, h; nucleotide diversity, π; and number of nucleotide differences, k) were performed using DNASP 3.0 (Rozas and Rozas 1997). Graphically, haplotype areas were visualized by 95% confidence ellipses for the coordinates of the respective samples. The SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and SYSTAT 12.0 (SYSTAT Software Inc., Richmond, CA, USA) statistical software packages were used for the statistical calculations. The significance level was set to α=0.05.

Habitat modelling

The distribution of great bustards in Spain was modelled at 1×1 km resolution from a set of predictor variables including vegetation, topography and human disturbance estimates. Vegetation was characterized by using a 12-month time-series of normalized difference vegetation indices (NDVI), calculated from NOAA-AVHRR satellite data. NDVI separates green vegetation from other surfaces because chlorophyll absorbs red light and reflects near infrared wavelengths. It may vary depending on land use, season and climate. Since regional differences in the timing of seasons and agricultural production can happen across Spain, we applied standardized principal components analysis (PCA) to replace the original 12-monthly variables with 12 independent components that may be interpreted as particular environmental features or events. Topographic variability was estimated in a 5×5 pixel array of 200-m pixels, where altitude was computed to 5-m vertical resolution. Human disturbances were measured as the proportion of 200-m pixels in a 5×5 array containing roads and towns and the distance in km to the nearest 200-m pixel containing those features. Also, proportion and distance to

rivers were included in the analysis. Relationships between occurrence bird data and environmental predictors were explored by applying generalized additive models (GAMs). We used S-plus 2000 (Venables and Ripley 1999) and the GRASP (Generalized Regression Analysis and Spatial Prediction) interface (Lehmann et al. 2003) to fit cubic splines with four degrees of freedom for each predictor, using a logit link and binomial error structure. Models were generated by means of a backwards variable selection at a significance level of 0.05 (Pearce and Ferrier 2000) testing, for each selected variable, significant differences between smooth and linear terms. Terms contribution to the model was assessed using a likelihood ratio test and model performance was evaluated through 10-fold cross-validation. Output model was a HSI ranging from 0 to 1 (Fig. 1), which can be used as an indicator of habitat quality for the species. The model fitted the data well, with an ROC score of 0.96, which reduced by only 0.01 when it was cross-validated, suggesting its robustness and predictability. HSI was produced in IDRISI 32.11 (Eastman 2000). For a more detailed explanation of the data sets and statistical methods used, see Suárez-Seoane et al. (2002). We then calculated the averaged HSI within a moving window of 3×3 km (i.e. 9 km^2; the minimum size of a lek) centered sequentially on each pixel corresponding to each sample location. We assigned these HSI mean values to the respective samples. Differences in habitat quality among haplotypes were tested by performing ANOVA.

Results

Spatial pattern in genetic diversity

We found 20 distinct mtDNA haplotypes, defined from 14 variable positions in 302 individuals (Table 1). The four of most frequent haplotypes (nos. 3, 5, 2 and 17 were present in respectively 34%, 15%, 13% and 8% of all individuals investigated. The overall nucleotide diversity (π), i.e. the percentage of the average number of nucleotide differences per site between two sequences (Nei 1978) was π=0.49%, while the overall haplotype diversity (h) was 0.83. The corresponding values within predefined regions are shown in Table 1.

Habitat quality and distribution

The habitat suitability map (Fig. 1) shows a highly diverse mosaic of habitat qualities at countrywide or regional scales, indicating that the HS model was able to discriminate between suitable and unsuitable habitats and that the set of eco-geographical variables allowed us distinguish specific habitats preferred by great bustards from the overall habitat

Table 1 Geographic distribution of mtDNA haplotypes, number of individuals (N), haplotype diversity (h), and nucleotide diversity (π) within predefined regions throughout the breeding range of the great bustard in the Iberian peninsula

Haplotype[a]	Accession no.	Variable sites[b] 58111122222445 70333744569392 35605248358	Albacete	Aragón	Burgos	Cáceres	Castilla	Córdoba	Huelva	Madrid N	MadridSW	MadridSE	Navarra	Sevilla	Toledo E	Toledo W	Villafáfila	N
H_1	AF421964	CTGCCTCACACGG							4	10	3	1			1	3		22
H_2	AF421966	CCGTCTTCACACGA	1			2	5			18	5	2			1		5	39
H_3	AF421967	CCGCCTCACGTAA					2			70	7	9	11		2		2	103
H_4	AF421974	TCGCCTCACACGA								3						1		4
H_5	AF422106	CCGCCCTACACGA	6	4	2		3	4		11	3	3			5		4	45
H_6	AF422007	CCGCCTCACGCGG							1	1								2
H_7	DQ445296	CCGTCTTTACACGA				2	2	1	4									9
H_8	AF422095	CCGCCTCCACGTAA				4	8				4	1		1		1		19
H_9	DQ445297	CCGCCTCACACGG			1	2	4							3	1	1		12
H_10	DQ445298	CCGCCTCTGCACGA				1												1
H_11	DQ445299	CCGCCTCACACGA				1								1			1	3
H_12	DQ445300	CCGTCTTCACATAA				1												1
H_13	AF422064	CCGCCCTACGTAA					4				1						1	6
H_14	AF422060	CCGCCTCCACACGA								1	2							3
H_15	DQ445301	CTGCCTCACACGA									1							1
H_16	DQ445302	CCGCCTCACACGA												2				2
H_17	DQ445303	CCGCCTCACGTGA						7	2					15				24
H_20	AF422100	CCGTCTCACACGA									1							1
H_21	DQ445304	CCGCCCCTATACGA						4										4
H_22	DQ445305	CCACCTCTACACGA	1															1
N			8	4	3	13	28	16	11	114	27	16	11	22	10	6	13	
h (%)			46.4	0.0	66.7	88.5	84.5	72.5	76.4	58.5	87.5	66.7	0.0	52.8	75.6	78.6	78.2	
π (%)			0.22	0.0	0.20	0.53	0.53	0.34	0.43	0.45	0.54	0.44	0.0	0.22	0.42	0.52	0.49	

[a] Note the absence of the haplotypes H_18 and H_19 not found in Spain

[b] The sequenced fragments correspond to positions 280–945 in the control region of the complete *Ciconia ciconia* mitochondrial genome (AB026818). Letters designate base substitutions. Nucleotide position numbers of the great bustard mtDNA correspond to positions in the alignment

*P<0.05

**P<0.01

n.d. Not determined because of limited sample size.
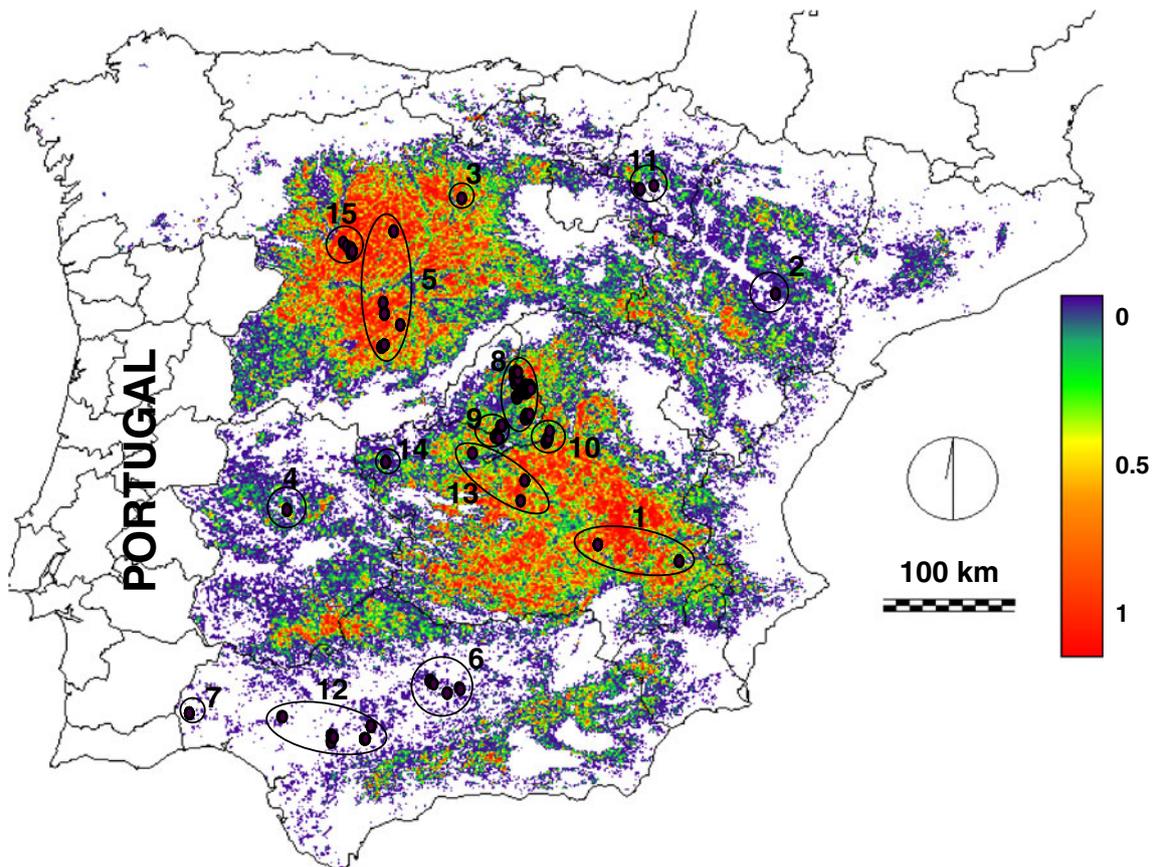
Fig. 1 Sample locations plotted on a map of suitable habitats for great bustard in Spain derived from Suárez-Seoane et al. (2002). The habitat suitable index ranges from 0 (unsuitable) to 1 (highly suitable). Predefined regions (*ellipses*) are: Albacete (*1*), Aragón (*2*), Burgos (*3*), Cáceres (*4*), Castilla (*5*), Córdoba (*6*), Huelva (*7*), Madrid N (*8*), Madrid SW (*9*), Madrid SE (*10*), Navarra (*11*), Sevilla (*12*), Toledo E (*13*), Toledo W (*14*), Villafáfila (*15*). Details on the distribution of the great bustard populations in Spain and Portugal can be found in the work of Alonso et al. (2003) and Pinto et al. (2005)

available in Spain. The highest-quality habitats were wide areas showing gentle terrain undulations covered by unobstructed cereal fields mosaics with a low diversity of strata, but a high herbaceous cover (intermediate values of overall greenness), which may offer adequate concealment for birds. Lowest-quality habitats were also steppe-like areas, but showing a higher diversity of strata with lower herbaceous cover (greenness) in more abrupt terrain, at higher altitude.

Based on the results of predictive habitat modelling, 83.3% of the entire area studied was defined as good-quality habitat for great bustards, with HSI values ranging from 0.62 (Cáceres) to 0.89 (Castilla), 7.5% of the area was of intermediate quality between 0.19 in Toledo West and 0.48 in Madrid South-West, and 9.3% was of poor-quality habitats, with HSI values not significantly different from zero (Córdoba, Sevilla and Huelva) (Table 2). According to our census data, 93.0% of the population represented by our genetic sample lives in good-quality habitats, while 4.5% and 2.5% occur respectively in intermediate and poor habitats.

The HSI differences between the geo-referenced haplotypes are illustrated in Fig. 2. Obviously, the haplotypes 16, 17 and

21 show extremely low habitat quality indices. In order to verify the potential spatial isolation of these three haplotype areas, we calculated spatial 95% confidence ellipses for the pooled haplotypes 16, 17, 21 and the pooled set of the remaining haplotypes based on the sampled individual's geographic coordinates (Fig. 3). In advance, the individuals had been weighted according to the population they belong to.

Correlating genetic variation with demographic variables and habitat quality

We conducted simple correlation analyses to investigate the relationships among nucleotide diversity (π), patch size, population size, population density, and habitat quality (i.e. HSI) (Table 3). Here, we included only populations with at least ten sampled individuals. Habitat quality was highly correlated with patch size ($\rho=0.829$, $P=0.002$), population size ($\rho=0.909$, $P<0.001$) and population density ($\rho=0.773$, $P=0.005$) indicating the reliability and predictive power of the habitat suitability model. Genetic diversity was significantly correlated with habitat quality ($\rho=0.620$, $p=0.042$),

Table 2 Habitat suitability index (HSI), area size, population size, and population density of the regions sampled in this study

Region	HSI		Patch size (km²)	Population size	Population density
	Mean	SD			
Albacete	0.657	0.1804	860	571	0.66
Aragón	0.3309	0.0000	250	66	0.26
Burgos	0.7854	0.0000	125	104	0.83
Cáceres	0.6234	0.0000	600	889	1.48
Castilla	0.8869	0.0785	2,414	2,870	1.19
Córdoba	0.0544	0.0423	198	79	0.40
Huelva	0.0030	0.0003	50	25	0.50
Madrid N	0.7091	0.1538	600	1,207	2.01
Madrid SE	0.4253	0.0596	56	65	1.16
Madrid SW	0.4769	0.1362	110	180	1.64
Navarra	0.2672	0.0997	68	30	0.44
Sevilla	0.0049	0.0047	460	139	0.30
Toledo E	0.6710	0.1236	561	770	1.37
Toledo W	0.1920	0.0000	85	100	1.18
Villafáfila	0.8662	0.0867	1,202	2,838	2.36
Total	0.5479	0.3038	7,639	9,933	

size ($\rho=0.624$, $p=0.040$) and density ($\rho=0.756$, $p=0.007$) of the population, but not with patch size ($\rho=0.436$, $p=0.180$). Among these variables, habitat quality was the only extrinsic factor potentially influencing the population development including population size and density. Therefore, partial rank correlation coefficients were calculated controlling for HSI. The resulting partial correlation coefficients regarding genetic diversity were not significant, as opposed to their non-partial equivalents (Table 3). These results suggest the HSI to be one of the underlying variables behind the pairwise correlations between genetic diversity and the population characteristics.

Discussion

Demographic and ecological correlates of genetic variability

Consistent with the classic island biogeography theory (Frankham 1996), i.e. that population size and density affect genetic variability, we found significant positive correlations between these demographic parameters and genetic variability. We hypothesized that variations in
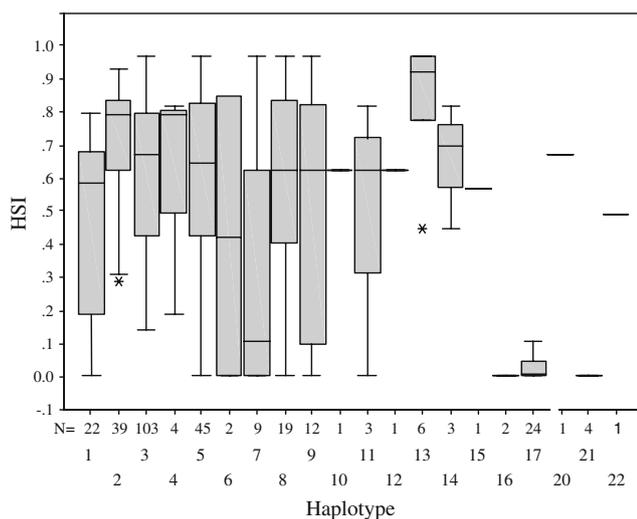
Fig. 2 Boxplot showing the median habitat suitability index (HSI) of each haplotype. *Horizontal lines*, median values; *boxes*, 25 and 75 percentiles; *vertical lines* include 10–90 percentiles; *stars*, outlier values
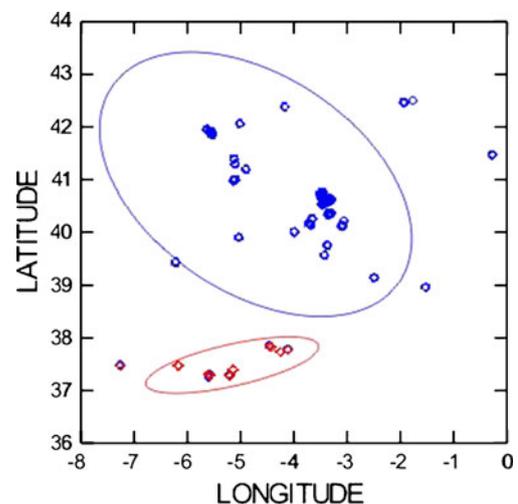
Fig. 3 The 95% ellipses for the pooled haplotypes 16, 17 and 21 (*rhombus-shaped*) and the pooled remaining haplotypes (*circles*). The ellipses are centred on the sample means of the respective longitudes and latitudes. The standard deviations of longitude and latitude determine the major axes, and the covariance between longitude and latitude, the ellipses' orientations

Table 3 Rank correlations of genetic diversity with habitat quality and demographic variables across great bustard study sites

	Genetic diversity	Patch size	Population size	Population density	Habitat quality
Genetic diversity	–	0.436	0.624*	0.756**	0.620*
Patch size	−0.211	–	0.961**	0.519	0.829**
Population size	0.191	0.890**	–	0.682[*]	0.909**
Population density	0.591	−0.342	−0.078	–	0.773**

Correlation coefficients (Spearman's r) above and partial rank correlation coefficients controlling for HSI below the diagonal. Populations in Albacete, Aragón, Burgos, and Toledo West were excluded because of their small sample size (<10 individuals)

*$P < 0.05$

** $P < 0.01$

habitat quality would have an influence on the amount and spatial distribution of genetic variation. Our results indeed confirmed that genetic diversity was significantly correlated with habitat quality. Although that correlation does not imply causation, it suggests that habitat quality may be an important factor determining changes in great bustard numbers, and also causing variations in the genetic diversity and structure of the population studied. We do not believe that this result could be influenced by two caveats concerning the partitioning of genetic diversity in our study. First, the estimate of genetic diversity at each study site is only a crude approximation due to the small sample sizes, implying that estimates of π might change with additional sampling. Second, the fact that we intentionally sampled several individuals in relatively small areas separated from each other by considerable distances, may have exaggerated the similarity within sample regions and the differences between regions.

Attempts to use measures of habitat quality to predict current genetic variation in wild populations are in their infancy. Thus far, they rarely appear to be able to explain the partitioning of diversity in natural populations (Angers et al. 1999; Costello et al. 2003). This might reflect our incomplete understanding of the complex nature of the relationship between habitat quality and population dynamics. Our findings of a significant positive correlation between HSI values and demographic data may be attributed to the selection of appropriate environmental variables to build the habitat suitability model for great bustards in Spain. Currently, a wide variety of methods for modelling species habitat suitability is available (Franklin and Miller 2009). Many of these methods require observations of species presence and absence, which should be widely distributed across space and environmental gradients, to characterize complex response functions (Guisan et al. 2002). Others are based on only-presence data (Elith et al. 2006; Pearce and Boyce 2006). In general terms, according to Brotons et al. (2004), when presence–absences are available, a modelling approach based on a binary response variable is preferable. The cause is that they

generally give more accurate predictions than models based on only-presence data. It should be noticed that one of the most reported troubles when dealing with these methods is the quality of absences, which use to be actually 'peudo-absences', mainly because of detectability problems. However, when absences are 'safe' (as it is the case of the species showing a well known distribution pattern, e.g. great bustard in Spain), the application of methods based in presences–absences become more powerful. Among 'presence–absences methods', GAM has been recommended as one of the best choices for species distribution modelling and spatial prediction because it tends to have high prediction accuracy, being a powerful tool able to model non-linear species responses curves (Wood 2006; Meynard and Quinn 2007).

HSI models can be valuable tools, but should always be applied with a critical evaluation of the model assumptions and uncertainties in relation to the research or management problem (Van der Lee et al. 2006). Despite their widespread use, HSI models can be justified only if they are tested against population measures such as density, reproductive success or genetic diversity and structure (Fulgione et al. 2009). In the future, similar comparative HSI-based ecological and genetic studies with other taxa would help assessing whether there is really a relationship between genetic variation and sensitivity to local habitat conditions, as our results suggest.

Implications for conservation

The recorded genetic diversity of $\pi = 0.49\%$ in Spain is similar to that of the great bustards in the Ponto-Caspian steppes (Ukraine and the Lower Volga basin) ($\pi = 0.68\%$; Pitra et al. 2007). Spanish great bustards form a metapopulation of several subpopulations with various degrees of interconnection among them (Martín et al. 2002; Alonso et al. 2009). The patch sizes occupied by these subpopulations are significantly correlated with the respective bird numbers. Because 92% of the total number of individuals live in large patches (>250 km^2) with good habitat quality, the demo-

graphic stability of the metapopulation seems to be secured, as long as habitat quality does not change in these patches. However, the finding that a substantial proportion of the total genetic diversity of Iberian great bustards is currently restricted to low-quality areas was of primary importance. We discuss these patterns and their likely implications for conservation in more detail below. The different Spanish regions have been subjected to various degrees of habitat deterioration mainly due to agricultural transformations within the last decades (Alonso et al. 2003, 2004). The results of our genetic study reveal that the existing genetic inventory of the Spanish population may be threatened if habitat deterioration proceeds. Based on the spatially non-random distribution of the present genetic variation, two risk factors for ongoing genetic erosion were identified: small size of some populations and poor quality of some habitats. For instance, the populations in Navarra and Aragón are very small (30 and 66 individuals) and show extremely low genetic diversity values (Table 1). These small and geneti-cally impoverished populations might suffer from inbreeding depression and increased probabilities of extinction (Frankham 1995). The populations in Andalusia (Huelva, Córdoba and Sevilla) show moderate genetic diversity values (0.22–0.43) but very low HSIs (0.003–0.054). These pop-ulations were much larger in the past, and the low HSI values reflect a poor habitat quality caused by agriculture intensifi-cation during last decades (Alonso et al. 2003, 2005). Since genetic diversity in neutral markers is lost at greater rates in smaller than larger populations (Montgomery et al. 2000), this could explain why populations in Andalusia would have reacted slowly to habitat changes. Lane and Alonso (2001) and Alonso et al. (2005) have reported local extinctions within the last two decades and population viability analyses under the status quo conditions inferred 85–99% extinction probability within 100 years for local breeding groups in Andalusia. Given the precarious status of the species in this region, our finding that three matrilineages (haplotypes 16, 17 and 21) are restricted to poor-quality habitats in Andalusia is alarming. Assuming the worst case scenario, the extinction of the Andalusian population would cause a loss of three (15%) of a total of 20 existing matrilineages from the species' current genetic pool in Spain. Consequently, strict conservation measures should be directed at populations located in low-quality habitats. Moreover, management actions should focus on the protection of local populations to promote conservation of the current genetic diversity and persistence of the metapopulation structure. The distribution of great bustards in Spain is highly fragmented, and vacant habitat patches may occur for a variety of reasons, including the size and quality of habitat areas as well as the species' very strong fidelity to traditional lek sites and conspecific attraction (Lane et al. 2001; Alonso et al. 2003, 2004). According to present knowledge, maintaining a dense network of suitable patches and maximizing local habitat conditions in order to improve reproductive output, and thus the number of potential dispersers, is the key to secure the persistence of the great bustard metapopulation in Spain and in other similarly fragmented landscapes.

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