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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** 39

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](http://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

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

82 In this context, it becomes particularly important to  
 83 quantify how variation in ecological factors linked to  
 84 habitat quality might affect and shape observed levels of  
 85 genetic variability in wild populations. The effects of  
 86 habitat loss and fragmentation on the genetic structure and  
 87 variability of wild populations have received wide empir-  
 88 ical support and theoretical formalization (Frankham 1995).  
 89 By contrast, the effects of habitat quality (or suitability)  
 90 seem largely underinvestigated, partly due to technical  
 91 difficulties in properly assessing habitat quality (Vergeer  
 92 et al. 2003). There are a large variety of methods in the  
 93 literature to measure it (see Johnson 2007 for a review),  
 94 either assessing directly habitat attributes or recording  
 95 variables from individuals/populations. Recent develop-  
 96 ments in geographic information systems (GIS) and  
 97 habitat modelling provide an ideal methodological frame-  
 98 work in which high-resolution analyses of habitat attrib-  
 99 utes can be performed at the landscape level. Models  
 100 allow gaining insights into the environmental variables  
 101 (such as biotic, physical, climatic or human-induced  
 102 habitat conditions) acting on species survival by compar-  
 103 ing current species distribution with the optimal values  
 104 for each variable (Guisan and Zimmermann 2000;  
 105 Osborne et al. 2001; Suárez-Seoane et al. 2002). The  
 106 output of the models is a habitat suitability index (HSI)  
 107 which ranges from 0 (low quality) to 1 (high quality). It  
 108 has been widely used since the early 1980s to determine

wildlife habitat quality (U.S. Fish and Wildlife Service 109  
 1980, 1981), as García et al. (2007), among other authors, 110  
 did for a fragmented population of little bustard in NW 111  
 Spain. This study examines the interactions between 112  
 habitat quality and genetic diversity in a range of natural 113  
 populations ( $n=15$ ) of *O. tarda* throughout the Spain and 114  
 considers the implications of the results for the species' 115  
 conservation. Specifically, we used a GIS-based distribu- 116  
 tion model previously developed for *O. tarda* in Spain to 117  
 infer a spatially explicit HSI capable of identifying, 118  
 categorizing and ranking landscape quality (Suárez- 119  
 Seoane et al. 2002). The spatial pattern of the species' 120  
 genetic diversity was then overlaid to address the 121  
 following objectives: (1) to investigate at a nationwide 122  
 scale the spatial patterns of genetic variation, (2) to 123  
 evaluate the possible influence of habitat quality and 124  
 some demographic variables (patch size, population size, 125  
 population density) on genetic variation and (3) to 126  
 identify those haplotypes and determine the proportion 127  
 of the overall genetic variation of the species in the study 128  
 area currently living under poor habitat conditions and 129  
 deserving high conservation priority. 130

## 131 Material and methods

### 132 Bird data and sample collection

133 Data about numbers and distribution of the species were 134  
 compiled from censuses carried out in Spain by different 135  
 teams of observers between mid March and early April 136  
 (1994–2008), when great bustards gather at their mating 137  
 areas and thus are easier to count (details and references in 138  
 the study of Alonso et al. 2003; Palacín and Alonso 2008). 139  
 A team consisted of two observers with experience in 140  
 counting bustards and knowledge of the area to be 141  
 surveyed. They drove four-wheel-drive vehicles slowly 142  
 through most tracks, trying to count all birds and to avoid 143  
 duplicated counts. Each census was carried out from dawn 144  
 to dusk, with a midday pause, when birds often sit down 145  
 and are harder to detect. Sampling regions were chosen to 146  
 represent the distribution range of the species in Spain 147  
 (Alonso et al. 2003). The corresponding population and 148  
 patch sizes were delimited according to discontinuities in 149  
 the distribution of both, birds and areas of steppe-like 150  
 habitat, considering the movements of a large sample of 151  
 radio-tagged individuals (more than 700 birds in total, 152  
 1991–2009, unpublished data). A total of 302 tissue 153  
 samples of blood, feathers or unfertilised eggs from various 154  
 Spanish regions were collected from breeding adults or 155  
 chicks throughout the great bustard breeding range in 156  
 Spain. The UTM coordinates were determined for each 157  
 sample using a GARMIN-12 GPS.

158	Genetic analysis		
159	DNA extraction, polymerase chain reaction (PCR) ampli-	208	
160	fication of a 657 base pair (bp) fragment of the mitochon-	209	
161	drial control region (Domain I=283 bp, Domain II=	210	
162	374 bp), and DNA sequencing were performed using the	211	
163	protocols outlined by Martín et al. (2002). The PCR	212	
164	products were purified (QIAquick PCR purification kit/	213	
165	Qiagen), sequenced by the extension-dideoxy-chain termi-	214	
166	nation method (Sanger et al. 1977) with a commercial kit	215	
167	(Dye Terminator Cycle Sequencing Kit, Applied Biosys-	216	
168	tems/Perkin Elmer) and analysed on a 373 A sequencer	217	
169	(Applied Biosystems). Sequences were aligned manually	218	
170	and all variable sites were confirmed by visual inspection of	219	
171	the chromatograms. Sequences of the same haplotype were	220	
172	identified using the computer program Collapse 1.1 (Posada	221	
173	and Crandall 1998). The resulting 20 different haplotypes	222	
174	were submitted to the GenBank database and included in	223	
175	the subsequent analysis (Table 1). Initial sequence compar-	224	
176	isons and measures of genetic variability (haplotype	225	
177	diversity, $h$ ; nucleotide diversity, $\pi$ ; and number of	226	
178	nucleotide differences, $k$ ) were performed using DNASP	227	
Q2 179	3.0 (Rozas and Rozas 1997). Graphically, haplotype areas	228	
180	were visualized by 95% confidence ellipses for the	229	
181	coordinates of the respective samples. The SPSS 16.0	230	
182	(SPSS Inc., Chicago, IL, USA) and SYSTAT 12.0	231	
183	(SYSTAT Software Inc., Richmond, CA, USA) statistical	232	
184	software packages were used for the statistical calculations.	233	
185	The significance level was set to $\alpha=0.05$ .	234	
186	Habitat modelling	235	
187	The distribution of great bustards in Spain was modelled at		
188	1×1 km resolution from a set of predictor variables		
189	including vegetation, topography and human disturbance		
190	estimates. Vegetation was characterized by using a 12-		
191	month time-series of normalized difference vegetation		
192	indices (NDVI), calculated from NOAA-AVHRR satellite		
193	data. NDVI separates green vegetation from other surfaces		
194	because chlorophyll absorbs red light and reflects near		
195	infrared wavelengths. It may vary depending on land use,		
196	season and climate. Since regional differences in the timing		
197	of seasons and agricultural production can happen across		
198	Spain, we applied standardized principal components		
199	analysis (PCA) to replace the original 12-monthly variables		
200	with 12 independent components that may be interpreted as		
201	particular environmental features or events. Topographic		
202	variability was estimated in a 5×5 pixel array of 200-m		
203	pixels, where altitude was computed to 5-m vertical		
204	resolution. Human disturbances were measured as the		
205	proportion of 200-m pixels in a 5×5 array containing roads		
206	and towns and the distance in km to the nearest 200-m pixel		
207	containing those features. Also, proportion and distance to		
	Q3 rivers were included in the analysis. Relationships between	208	
	occurrence bird data and environmental predictors were	209	
	explored by applying generalized additive models (GAMs).	210	
	We used S-plus 2000 (Venables and Ripley 1999) and the	211	
	GRASP (Generalized Regression Analysis and Spatial	212	
	Prediction) interface (Lehmann et al. 2003) to fit cubic	213	
	splines with four degrees of freedom for each predictor,	214	
	using a logit link and binomial error structure. Models were	215	
	generated by means of a backwards variable selection at a	216	
	significance level of 0.05 (Pearce and Ferrier 2000) testing,	217	
	for each selected variable, significant differences between	218	
	smooth and linear terms. Terms contribution to the model	219	
	was assessed using a likelihood ratio test and model	220	
	performance was evaluated through 10-fold cross-	221	
	validation. Output model was a HSI ranging from 0 to 1	222	
	(Fig. 1), which can be used as an indicator of habitat quality	223	
	for the species. The model fitted the data well, with an ROC	224	
	score of 0.96, which reduced by only 0.01 when it was	225	
	cross-validated, suggesting its robustness and predictability.	226	
	HSI was produced in IDRISI 32.11 (Eastman 2000). For a	227	
	more detailed explanation of the data sets and statistical	228	
	methods used, see Suárez-Seoane et al. (2002). We then	229	
	calculated the averaged HSI within a moving window of	230	
	3×3 km (i.e. 9 km <sup>2</sup> ; the minimum size of a lek) centered	231	
	sequentially on each pixel corresponding to each sample	232	
	location. We assigned these HSI mean values to the	233	
	respective samples. Differences in habitat quality among	234	
	haplotypes were tested by performing ANOVA.	235	
	<b>Results</b>	236	
	Spatial pattern in genetic diversity	237	
	We found 20 distinct mtDNA haplotypes, defined from 14	238	
	variable positions in 302 individuals (Table 1). The four of	239	
	most frequent haplotypes (nos. 3, 5, 2 and 17 were present	240	
	in respectively 34%, 15%, 13% and 8% of all individuals	241	
	investigated. The overall nucleotide diversity ( $\pi$ ), i.e. the	242	
	percentage of the average number of nucleotide differences	243	
	per site between two sequences (Nei 1978) was $\pi=0.49\%$ ,	244	Q3
	while the overall haplotype diversity ( $h$ ) was 0.83. The	245	
	corresponding values within predefined regions are shown	246	
	in Table 1.	247	
	Habitat quality and distribution	248	
	The habitat suitability map (Fig. 1) shows a highly diverse	249	
	mosaic of habitat qualities at countrywide or regional scales,	250	
	indicating that the HS model was able to discriminate	251	
	between suitable and unsuitable habitats and that the set of	252	
	eco-geographical variables allowed us distinguish specific	253	
	habitats preferred by great bustards from the overall habitat	254	

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

Haplotype <sup>a</sup>	Variable sites <sup>b</sup>	Accession no.	Albacete	Aragón	Burgos	Cáceres	Castilla	Córdoba	Huelva	Madrid N	MadridSW	MadridSE	Navarra	Sevilla	Toledo E	Toledo W	Villafañila	<i>N</i>
t1.3		5811112222445 70333744569392 356056248358																
t1.4		CTGCCCTACACGG AF421964						4		10	3	1			1	3		22
t1.5		CCGTCTTACACGA AF421966	1		2	5	2			18	5	2			1		5	39
t1.6		CCGCCCTACGTAA AF421967			2					70	7	9	11		2		2	103
t1.7		TCGCCCTACACGA AF421974								3					1		4	45
t1.8		CCGCCCTACACGA AF422106	6	4	2	3	4	4		11	3	3			5		4	2
t1.9		CCGCCCTACGCGG AF422007							1	1								9
t1.10		CCGTCTTACACGA DQ445296			2	2	1	4										19
t1.11		CCGCCCTCCACGTAA AF422095			4	8	4			4	1	1		1	1			12
t1.12		CCGCCCTACACGG DQ445297		1	2	4								3	1			1
t1.13		CCGCCCTGCACGA DQ445298			1									1		1		3
t1.14		CCGCCCTACACGA DQ445299			1									1				1
t1.15		CCGTCTTACACATAA DQ445300																6
t1.16		CCGCCCTACGTAA AF422064				4					1						1	3
t1.17		CCGCCCTCCACACGA AF422060								1	2							1
t1.18		CTGCCCTACACGA DQ445301									1							2
t1.19		CCGCCCTACACGA DQ445302												2				24
t1.20		CCGCCCTACGTGA DQ445303					7	2						15				1
t1.21		CCGTCTTACACGA AF422100									1							1
t1.22		CCGCCCTTACACGA DQ445304					4											4
t1.23		CCACCTTACACGA DQ445305	1															1
t1.24			8	4	3	13	28	16	11	114	27	16	11	22	10	6	13	
t1.25	<i>h</i> (%)		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	
t1.26	$\pi$ (%)		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	

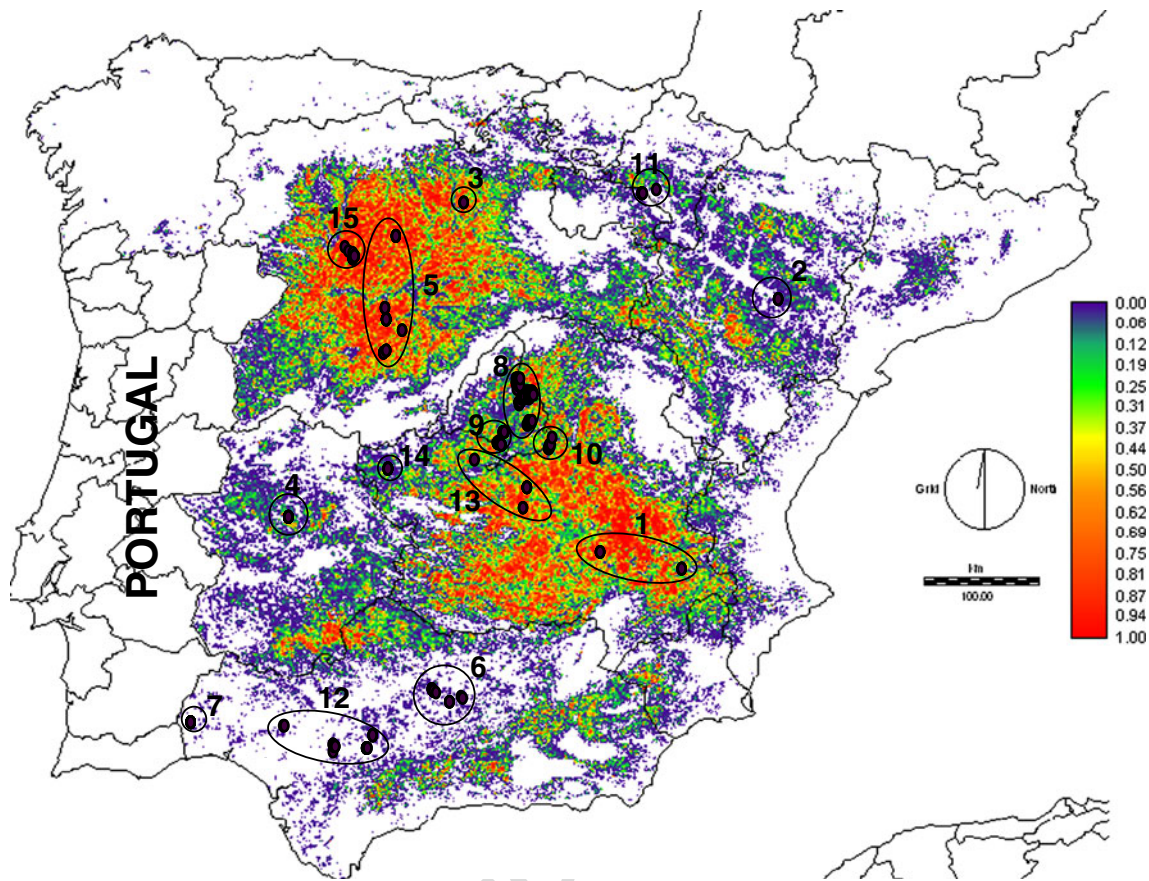
<sup>a</sup> Note the absence of the haplotypes H\_18 and H\_19 not found in Spain

<sup>b</sup> 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 suitability 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)

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

263 Based on the results of predictive habitat modelling, 83.3%  
 264 of the entire area studied was defined as good-quality habitat  
 265 for great bustards, with HSI values ranging from 0.62  
 266 (Cáceres) to 0.89 (Castilla), 7.5% of the area was of  
 267 intermediate quality between 0.19 in Toledo West and 0.48  
 268 in Madrid South-West, and 9.3% was of poor-quality habitats,  
 269 with HSI values not significantly different from zero  
 270 (Córdoba, Sevilla and Huelva) (Table 2). According to our  
 271 census data, 93.0% of the population represented by our  
 272 genetic sample lives in good-quality habitats, while 4.5% and  
 273 2.5% occur respectively in intermediate and poor habitats.

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

21 show extremely low habitat quality indices. In order to  
 276 verify the potential spatial isolation of these three haplotype  
 277 areas, we calculated spatial 95% confidence ellipses for the  
 278 pooled haplotypes 16, 17, 21 and the pooled set of the  
 279 remaining haplotypes based on the sampled individual's  
 280 geographic coordinates (Fig. 3). In advance, the individuals  
 281 had been weighted according to the population they belong to.  
 282

Correlating genetic variation with demographic variables  
 and habitat quality 283 284

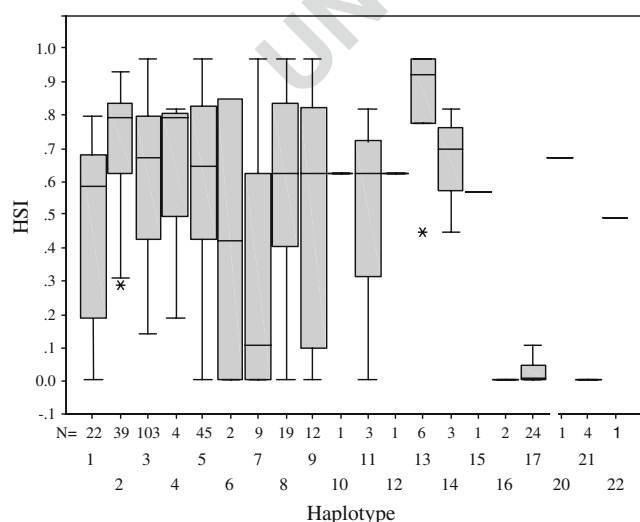
285 We conducted simple correlation analyses to investigate the  
 286 relationships among nucleotide diversity ( $\pi$ ), patch size,  
 287 population size, population density, and habitat quality (i.e.  
 288 HSI) (Table 3). Here, we included only populations with at  
 289 least ten sampled individuals.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

t2.1

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

Region	HSI		Patch size (km <sup>2</sup> )	Population size	Population density	
	Mean	SD				
Albacete	0.657	0.1804	860	571	0.66	t2.4
Aragón	0.3309	0.0000	250	66	0.26	t2.5
Burgos	0.7854	0.0000	125	104	0.83	t2.6
Cáceres	0.6234	0.0000	600	889	1.48	t2.7
Castilla	0.8869	0.0785	2,414	2,870	1.19	t2.8
Córdoba	0.0544	0.0423	198	79	0.40	t2.9
Huelva	0.0030	0.0003	50	25	0.50	t2.10
Madrid N	0.7091	0.1538	600	1,207	2.01	t2.11
Madrid SE	0.4253	0.0596	56	65	1.16	t2.12
Madrid SW	0.4769	0.1362	110	180	1.64	t2.13
Navarra	0.2672	0.0997	68	30	0.44	t2.14
Sevilla	0.0049	0.0047	460	139	0.30	t2.15
Toledo E	0.6710	0.1236	561	770	1.37	t2.16
Toledo W	0.1920	0.0000	85	100	1.18	t2.17
Villafáfila	0.8662	0.0867	1,202	2,838	2.36	t2.18
Total	0.5479	0.3038	7,639	9,933		t2.19

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.

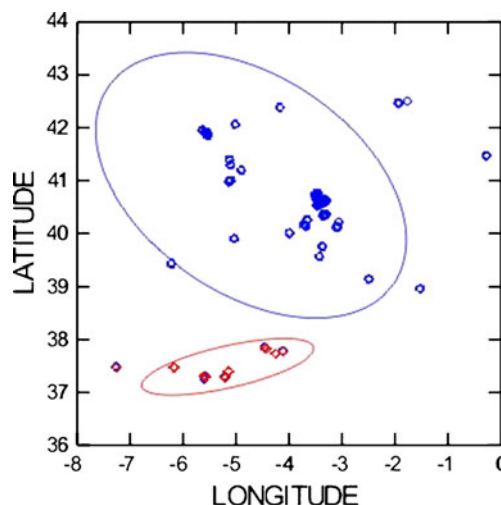


**Fig. 2** Boxplot showing the median habitat suitability index (HSI) of each haplotype. Horizontal lines, median values; boxes, 25 and 75 percentiles; vertical lines include 10–90 percentiles; stars, outlier values

## 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. 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	
t3.3	Genetic diversity	–	0.436	0.624*	0.756**	0.620*
t3.4	Patch size	–0.211	–	0.961**	0.519	0.829**
t3.5	Population size	0.191	0.890**	–	0.682*	0.909**
t3.6	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

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

333 Attempts to use measures of habitat quality to predict  
 334 current genetic variation in wild populations are in their  
 335 infancy. Thus far, they rarely appear to be able to explain  
 336 the partitioning of diversity in natural populations (Angers  
 337 et al. 1999; Costello et al. 2003). This might reflect our  
 338 incomplete understanding of the complex nature of the  
 339 relationship between habitat quality and population dynam-  
 340 ics. Our findings of a significant positive correlation  
 341 between HSI values and demographic data may be  
 342 attributed to the selection of appropriate environmental  
 343 variables to build the habitat suitability model for great  
 344 bustards in Spain. Currently, a wide variety of methods for  
 345 modelling species habitat suitability is available (Franklin  
 346 and Miller 2009). Many of these methods require observa-  
 347 tions of species presence and absence, which should be  
 348 widely distributed across space and environmental gra-  
 349 dients, to characterize complex response functions (Guisan  
 350 et al. 2002). Others are based on only-presence data (Elith  
 351 et al. 2006; Pearce and Boyce 2006). In general terms,  
 352 according to Brotons et al. (2004), when presence-absences  
 353 are available, a modelling approach based on a binary  
 354 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 'pseudo-  
 absences', mainly because of detectability problems. How-  
 ever, 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 'pres-  
 ence-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 metapopu-  
 lation 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<sup>2</sup>) with good habitat quality, the demo-

393 graphic stability of the metapopulation seems to be secured,  
 394 as long as habitat quality does not change in these patches.  
 395 However, the finding that a substantial proportion of the total  
 396 genetic diversity of Iberian great bustards is currently  
 397 restricted to low-quality areas was of primary importance.  
 398 We discuss these patterns and their likely implications for  
 399 conservation in more detail below. The different Spanish  
 400 regions have been subjected to various degrees of habitat  
 401 deterioration mainly due to agricultural transformations  
 402 within the last decades (Alonso et al. 2003, 2004). The  
 403 results of our genetic study reveal that the existing genetic  
 404 inventory of the Spanish population may be threatened if  
 405 habitat deterioration proceeds. Based on the spatially non-  
 406 random distribution of the present genetic variation, two risk  
 407 factors for ongoing genetic erosion were identified: small  
 408 size of some populations and poor quality of some habitats.  
 409 For instance, the populations in Navarra and Aragón are very  
 410 small (30 and 66 individuals) and show extremely low  
 411 genetic diversity values (Table 1). These small and genet-  
 412 ically impoverished populations might suffer from inbreeding  
 413 depression and increased probabilities of extinction  
 414 (Frankham 1995). The populations in Andalusia (Huelva,  
 415 Córdoba and Sevilla) show moderate genetic diversity values  
 416 (0.22–0.43) but very low HSIs (0.003–0.054). These pop-  
 417 ulations were much larger in the past, and the low HSI values  
 418 reflect a poor habitat quality caused by agriculture intensifi-  
 419 cation during last decades (Alonso et al. 2003, 2005). Since  
 420 genetic diversity in neutral markers is lost at greater rates in  
 421 smaller than larger populations (Montgomery et al. 2000),  
 422 this could explain why populations in Andalusia would have  
 423 reacted slowly to habitat changes. Lane and Alonso (2001)  
 424 and Alonso et al. (2005) have reported local extinctions  
 425 within the last two decades and population viability analyses  
 426 under the status quo conditions inferred 85–99% extinction  
 427 probability within 100 years for local breeding groups in  
 428 Andalusia. Given the precarious status of the species in this  
 429 region, our finding that three matrilineages (haplotypes 16,  
 430 17 and 21) are restricted to poor-quality habitats in Andalusia  
 431 is alarming. Assuming the worst case scenario, the extinction  
 432 of the Andalusian population would cause a loss of three  
 433 (15%) of a total of 20 existing matrilineages from the  
 434 species' current genetic pool in Spain. Consequently, strict  
 435 conservation measures should be directed at populations  
 436 located in low-quality habitats. Moreover, management  
 437 actions should focus on the protection of local populations  
 438 to promote conservation of the current genetic diversity and  
 439 persistence of the metapopulation structure. The distribution  
 440 of great bustards in Spain is highly fragmented, and vacant  
 441 habitat patches may occur for a variety of reasons, including  
 442 the size and quality of habitat areas as well as the species'  
 443 very strong fidelity to traditional lek sites and conspecific  
 444 attraction (Lane et al. 2001; Alonso et al. 2003, 2004).  
 445 According to present knowledge, maintaining a dense

network of suitable patches and maximizing local habitat 446  
 conditions in order to improve reproductive output, and thus 447  
 the number of potential dispersers, is the key to secure the 448  
 persistence of the great bustard metapopulation in Spain and 449  
 in other similarly fragmented landscapes. 450

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