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Consequences of Eagle Owl nest-site habitat preference for breeding performance and territory stability

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I tested the hypothesis that nest-site habitat preferences are adaptive in an Eagle Owl (*Bubo bubo*) population from Central Spain. Eagle Owls preferred to settle in areas linked with watercourses and with more irregular topography (physiographic gradient) and cover of dehesas and Mediterranean forests (vegetation gradient), whereas they avoided sources of human disturbances (human use gradient). Eagle Owls nesting in preferred positions along the physiographic gradient suffered lower clutch loss, probably due to reduced predation. Pairs settled in preferred habitats produced higher quality fledglings but not more fledglings. Diet analysis suggested that directional selection on fledgling quality could be partially due to better foraging performance in preferred habitats. Territories located in non-preferred habitats showed lower stability, suggesting an adaptive response to spatial variation in fitness that may be mediated by high rates of adult mortality or of breeding dispersal in non-preferred habitat. Overall, these results suggest that habitat preferences are adaptive in the Eagle Owl study population and conservation policies should be focused on protecting preferred habitats.

1. Introduction

A disproportionate occupancy of habitats, higher or lower use than average availability, has been reported for a large number of species (Clark & Shutler 1999). The process of natural selection which rules the observed habitat preference patterns is presumed to be related to changes in fitness among habitats (Cody 1985), a fact which is likely to lead to long-term selection for individuals occupying higher quality habitat (e.g. Martin 1998, Clark & Shutler 1999). This should form a basis for the evolution of habitat preferences (Clark & Shutler 1999). Hence, if habitat preferences are adaptive, individuals settled in the most preferred habitats should show higher fitness values and this step should be governed by natural selection, which will maintain the preferences if they have a genetic basis (Jaenike & Holt 1991).

In birds, several studies have analyzed correlates between habitat features and breeding performance or have looked for habitat differences between successful and unsuccessful nests. However, explicit tests of habitat preference adaptiveness, although rising in number, are less frequent in the literature (Clark & Shutler 1999, Sergio *et al.* 2003). To identify the habitats where individuals reach high fitness values is relevant itself, but only by exploring the adaptiveness of habitat choice can we understand whether individuals are attracted to areas where their fitness is improved, reduced (i.e. they suffer an ecological trap) or equal to that of individuals settled in non-preferred habitats. This allows assessing whether most preferred and most adequate habitats concur in space as well as predicting temporal changes in habitat use (Sergio *et al.* 2003). Thus, studying the adaptiveness of habitat preferences has important implications for both ecology and conservation research (Sergio *et al.* 2003).

Here I study nest-site habitat preferences in a large top predator, the Eagle Owl (*Bubo bubo*), under the conceptual framework of natural selection. For this purpose, I follow a sequential step procedure initially established by Clark and Shutler (1999) and developed by Sergio *et al*. (2003). Following such an approach, the main aims of this study are four-fold. 1) Establish the pattern of habitat preferences in the study Eagle Owl population, analyzing differences between used and available habitat features along different environmental gradients (long-term pattern resulted from natural selection). 2) Analyze whether different components of fitness (clutch size, frequency of clutch loss, number of fledglings and chick quality) vary along habitat preference gradients (the process of natural selection ruling the observed pattern). 3) Examine adaptive responses to spatial variation in fitness, by studying the stability of Eagle Owl territories in relation to habitat preferences. 4) Explore which selective agents could be responsible for natural selection and cause fitness differences.

2. Methods

2.1. Study species

The Eagle Owl is a top predator widely distributed in Eurasia. It is monogamous, territorial and sedentary (Snow & Perrins 1998). The species shows high plasticity and occupies several habitat types (Marchesi *et al.* 2002, Ortego & Díaz 2004). In Mediterranean populations the species reaches the highest population densities, due to high abundance of adequate prey in this region (Ortego & Díaz 2004). Studies analyzing habitat preferences in Eagle Owls have established that both physiographic and landscape structure parameters are decisive factors influencing its settlement (Penteriani *et al.* 2001, Marchesi *et al.* 2002, Martínez *et al.* 2003, Sergio *et al.* 2004, Ortego & Díaz 2004). These habitat clues have been generally interpreted as playing a role for both the protection of nest placement and foraging performance, factors which are recognized to affect different aspects of the species' reproductive performance (Marchesi *et al.* 2002, Penteriani *et al.* 2002, Penteriani *et al.* 2004, Sergio *et al.* 2004).

2.2. Study area

The study area (Toledo province, central Spain, 39º47'N, 4º04'W) covers 2,400 km^2 with meso-Mediterranean climate, mean temperatures ranging from 26ºC in July to 5ºC in January and 300–400 mm of rainfall concentrated in spring and autumn. The area is extensively cultivated, with irrigated maize *Zea mays* fields close to the Tajus river and non-irrigated barley *Hordeum vulgare* and wheat *Triticum* spp. fields, as well as scattered olive groves *Olea europaea* and vineyards *Vitis vinifera* elsewhere. Holm oaks *Quercus ilex* dominate the less intensively used areas, whereas the most altered zones are dominated by esparto grass *Stipa tenacissima* or Mediterranean scrubland mainly composed by *Quercus ilex* shrubs, *Cistus ladanifer* and *Retama sphaerocarpa*. Other minor habitats include watercourses with riparian vegetation and recent pine *Pinus* spp. plantations. Wild rabbits (*Oryctolagus cuniculus*), the main local prey for Eagle Owls in Mediterranean Spain (Serrano 2000, Martínez & Zuberogoitia 2001), show in the study area one of the highest population densities reported for the Iberian Peninsula (Villafuerte *et al.* 1995).

2.3. Field surveys

During 1999–2003 collaborators and I systematically censused Eagle Owl reproductive pairs in the study area (see Ortego & Díaz 2004 for census details). I monitored nests from March to early June during the 2001–2005 breeding seasons. I measured breeding parameters in selected Eagle Owl pairs along habitat preference gradients (see "statistical analyses" section for habitat preference study) previously developed to: 1) optimize the sampling effort, given that Eagle Owl territories are usually highly inaccessible in the study area, 2) reduce the disturbance on the study population, and 3) cover the total range of the habitat preference gradients obtained. For all study years I recorded laying date, clutch size (data from 30 territory-years; range 4–8 territories per year), clutch success/loss (data from 46 territory-years; range 5–16 territories per year), and number of fledged chicks (data from 114 territory-years; range 6–42 territories per year). However, only for 2004, chick residual mass and heterophil/lymphocyte ratio were determined (83 fledglings from 27 nests). Nests were visited at least two times in the chick nesting period. In the first visit, I calculated the age of the chicks according to their feather development by means of previous information from 11 nests containing chicks with exact hatching dates known (Marchesi *et al.* 2002, Penteriani *et al.* 2005). Laying date was calculated by subtracting 35 days, the average incubation period reported for this species (Snow & Perrins 1998), from the hatching date of the oldest sibling from each nest. The second visit was done when chicks were 40 days old to record the number of fledged chicks and, in 2004, to determine fledgling condition and obtain blood samples. This age was chosen because older fledgling Eagle Owls start to move around the nesting site, making them difficult to locate (Marchesi *et al.* 2002). Furthermore, brood reduction or deaths by starvation later than this age have not been reported in the study area (J. Ortego unpublished data).

I weighed fledglings with a 2.5 kg Pesola scale with a precision of 10 g, and took three linear measures of size (tarsus, back claw and bill lengths) using a caliper to the nearest 0.01 mm. To determine a body size index for the chicks, I carried out a principal component analysis using as input variables the three size measures taken ($n = 82$ chicks). A physical condition index (PCI) was then calculated for each bird using the residuals from a regression of body mass on the scores from the PC1 obtained (see Ortego & Espada 2007 for details).

I collected blood by puncturing the brachial vein of fledglings and transferred it to heparinized microcapillary tubes. Immediately, a drop of blood was smeared on four individually marked microscope slides. Each smear was rapidly air dried, fixed with absolute methanol and stained in the laboratory with Giemsa's solution (1:10) for 45 minutes. The proportion of different types of leucocytes (heterophils, eosinophils, basophiles, lymphocytes and monocytes) was assessed on the basis of a count of 100 leucocytes under oil immersion at 1,000× magnification (e.g. Ortego & Espada 2007). For the analysis I used the heterophil/lymphocyte ratio as an indicator of physiological stress (Gross & Siegel 1983, Maxwell & Robertson 1998) as its levels are known to rise in response to starvation in wild birds (Moreno *et al.* 2002). These methods have been previously reported to be highly repeatable (Saino *et al.* 1995). The person examining blood samples (F. Espada) had no information about individual birds except ring number.

Eagle Owls feed preferentially on wild rabbits within their Mediterranean distribution range, only switching to other alternative prey species when rabbits are scarce (Serrano 2000, Martínez & Zuberogoitia 2001). Thus, rabbit occurrence in the Eagle Owl diet has been suggested as a good indicator of their local availability (Serrano 2000). During 2004–2005, I estimated the diet of Eagle Owl chicks by counting the number of corpses (only entire prey were quantified) of each prey species found stored in the nests during repeated visits (2–4) during the nesting period (data from 46 territory-years; range 13–33 territories per year). Using these data, I determined the occurrence of rabbits in the chick diet as a measure of local rabbit availability.

I studied territory stability by checking adult presence or reproduction in previously occupied territories. I considered that a territory disappeared when no fresh-pellets, prey remains, recent molted feathers or evidence of reproduction were confirmed during two consecutive years (2004–2005). The studied pairs were not banded, but in Eagle Owls both life-span (15–20 years) and mate fidelity is likely to be high (Snow & Perrins 1998, Penteriani *et al.* 2004) and I suspect that pair members from stable territories did not change during the five years of study.

2.4. Habitat measurements

I measured 21 habitat variables within a circle of 1,500 m (7.07 km^2) radius around 115 nest locations and 100 random points obtained by contingent generation of a number of pairs of UTM coordinates. Given the high breeding density of Eagle Owls in the study area (Ortego 2004), neighboring occupied plots overlapped on average 28%. The spatial scale was selected according to previous studies which have defined this area as relevant for Eagle Owl nest-site habitats (Martínez *et al.* 2003, Ortego & Díaz 2004, Sergio *et al.* 2004). Furthermore, in areas with high prey availability like central Spain (Villafuerte *et al.* 1995), the studied spatial scale is expected to include most hunting points for parental Eagle Owls (Ortego & Díaz 2004), especially during the breeding season (Haller 1978, Leditznig 1996). Selected variables were related to sources of human disturbance, land uses and physiography (Appendix), parameters which have been previously defined as critical for Eagle Owl settlement and breeding performance (Marchesi *et al.* 2002, Penteriani *et al.* 2002, Martínez *et al.* 2003, Ortego & Díaz 2004, Penteriani et. al. 2004, Sergio *et al.* 2004). I determined distances, lengths, the index of topographic irregularity, the number of buildings and the density of unpaved roads in 1:25 000 topographic maps of Spain (I.G.N.). Nest sites were incorporated into a Geographic Information System (GIS) and, afterwards, the cover of land uses and the number of ecotones were measured in the digitalized 1:100,000 Corine Land Cover maps using the ArcView software (ArcView 3.2, ESRI, Redlands, California, USA). The 25 land use types provided by Corine Land Cover maps were grouped into nine categories to facilitate statistical analyses (Appendix).

2.5. Statistical analyses

2.5.1. Habitat preference patterns

I used a principal component analysis (PCA) to obtain environmental gradients and summarize and control for co-varying patterns in the 21 habitat variables measured both at nest sites and random points. Habitat variables were transformed for normality prior to the analysis using log, arcsine or root-square transformations. Although some variables did not become normally distributed after transformation, PCA analyses are robust to violations of the assumption of normality. The axes which followed the broken-stick criterion were retained (Jackson 1993) and then I extracted principal component scores and tested whether these scores differed between nests and random points by means of a logistic regression analysis (SAS Institute 2004). Quadratic terms were also analyzed to test possible non-linear relationships.

2.5.2. Fitness consequences of habitat preferences

To examine whether clutch loss and both clutch and brood size vary along habitat preference gradients (i.e. those principal components in which the score positions of nests and random points differed) I performed generalized linear mixed models (GLMM) using the GLIMIX macro of SAS (SAS Institute 2004). Apart from habitat preference gradients, I included laying date and nearest neighbor distance (NND) as covariates due to their potential influence on the species breeding performance (Marchesi *et al.* 2002, Penteriani *et al.* 2002, Penteriani 2003, Penteriani *et al.* 2004). Since some territories were monitored across years I fitted territory identity and year as random factors. I implemented GLMMs with Poisson distribution of errors and log link function for raw count data such as clutch and brood size. Clutch loss (presence/absence of the event) was analyzed using a GLMM with binomial error and logit link. As previously indicated, I calculated laying dates according to chick development so it could not be determined for those nests that lost the clutch and laying date was not included as a covariate in this analysis. In any case, laying date is unlikely to substantially affect clutch loss, a factor which is generally associated with predation risk in birds (Martin 1998). Residual body mass and heterophil/lymphocyte ratio were analyzed using a GLMM with normal error and identity link function, including habitat preference gradients, laying date and NND as covariates. I also included brood size as a predictor in these analyses, due to its potential negative influence on chick condition (e.g. Balbontin & Ferrer 2005), and nest identity was included as a random factor.

I analyzed territory stability along habitat preference gradients using a logistic regression (binomial error and logit link function). NND was also included as a predictor in this analysis. Another lo-

Table 1. Factor loadings, total and cumulative percent variance explained for the principal component analysis (VARIMAX normalized rotation) of habitat features measured in 115 Eagle Owl nests and 100 random points. Factor loadings with absolute values > 0.4 are shown in bold.

Habitat variables	Component			
	I	II	III	IV
Distance to village	–**0.69**	+0.17	–0.14	+0.30
Number of buildings	+**0.82**	+0.02	–0.05	–0.15
Distance to building	–**0.51**	–0.02	+0.17	+0.32
Distance to road	–**0.77**	+0.20	+0.17	–0.18
Distance to unpaved road	–0.06	+0.12	–0.03	+0.08
Length of paved roads	+**0.73**	–0.23	–0.21	+0.18
Density of unpaved roads	+0.27	–0.14	–0.10	–0.14
Non-irrigated herbaceous crops	+0.04	–0.39	+0.24	–0.35
Irrigated crops	+0.05	–0.07	–**0.88**	–0.01
Perennial crops	+0.14	–0.29	+0.16	–0.20
Tree plantations	+0.14	–0.03	–0.16	+0.04
Dehesa	–0.05	+0.06	–0.05	+**0.65**
Pasture	–0.03	–0.05	+0.07	–0.18
Scrubland	–0.10	+**0.50**	+0.03	–**0.53**
Mediterranean forest	–0.16	+0.35	+0.00	+**0.80**
Water	+0.08	+0.01	–**0.83**	+0.04
Urbanized areas	+**0.72**	+0.14	+0.11	–0.11
Ecotone number	+0.00	+0.21	–**0.49**	+0.03
Irregular topographic index	-0.22	+**0.57**	+0.09	+0.35
Distance to watercourse	+0.01	–**0.75**	+0.08	–0.12
Length of watercourses	-0.08	+**0.79**	–0.03	+0.06
Eigenvalue	4.74	2.52	1.77	1.61
Percent total variance	22.61	12.00	8.47	7.66
Cumulative variance	22.61	34.61	43.08	50.74

gistic regression was used to analyze whether the frequency of clutch failure (number of years in which a pair lost the clutch/number of years in which that pair was monitored) influences territory abandonment. The occurrence of wild rabbits in the diet of Eagle Owl chicks along habitat preference gradients was examined by performing a GLMM with binomial distribution of errors and logit link function. Instead of using the proportion of rabbits as a dependent variable which loses information on the sample size from which the occurrence of rabbits in the diet is estimated, I included the number of rabbits found stored in a given nest during the chick nesting period as the response variable and the total number of prey items found in that nest as binomial denominator. Since two years were studied (2004–2005) I included year and territory identity as random factors in this analysis.

To fine-tune the predictions according to the kind of selection occurring I included in all these analyses quadratic terms of habitat preference gradients (Endler 1986; e.g. Clark & Shutler 1999, Sergio *et al.* 2003). If pairs settled in preferred habitats have higher fitness values, then linear and positive relationships between fitness related parameters and habitat preference gradients will be obtained, suggesting directional selection. A negative quadratic relationship between fitness related parameters and habitat preference gradients will result when pairs settled at extremes of the gradient have lower fitness values, suggesting disruptive selection. Finally, a positive quadratic relationship will be obtained when pairs settled at extremes of habitat preference gradients show higher fitness values, suggesting stabilizing selection. Squared terms of habitat preference gradients were also included when I analyzed the contribution of rabbits to the Eagle Owl diet, since the manner in which this variable is associated with habitat gradients can be informative of the kind of selection it could exert.

All regression analyses in this study were performed initially fitting full models containing all explanatory variables. Final models were selected following a backward procedure, by progressively eliminating non-significant variables ($P < 0.1$). The significance of the remaining variables was tested again until no additional variable reached significance. The result is the most adequate model for explaining the variability in the response variable, where only the significant explanatory variables are retained. In GLMMs the significance of explanatory variables was determined using F-statistics for fixed effects and Z-statistics for random effects. In all other models not including random factors, I tested hypotheses using χ^2-statistics. All P-values refer to two tailed tests. In GLMMs I used a Satterthwaite correction which can result in denominator degrees of freedom that are not whole integers.

3. Results

3.1. Habitat gradients

The PCA analysis for habitat features yielded four factors which collectively accounted for 51% of the total variance (Table 1). The first component (PC1) was interpreted as a gradient related to human use, with positive loadings for number of buildings, length of paved roads and cover of urban areas and negative loadings for distance to buildings and roads. The second component (PC2) clearly defined the physiographical characteristics of the sampling points, separating areas of high topographic irregularity, with abundant valleys floors and high cover of scrublands (positive scores) from those areas distanced from watercourses (negative scores). The third component (PC3) grouped areas with high cover of irrigated crops and water bodies and elevated number of ecotones (negative scores), defining humanized and heterogeneous areas with land uses linked to water presence. The fourth component (PC4) represented a vegetation gradient, segregating areas of low altered natural vegetation formations (Mediterranean forest and dehesas) with positive scores from areas with more altered landscapes covered with scrublands (negative scores).

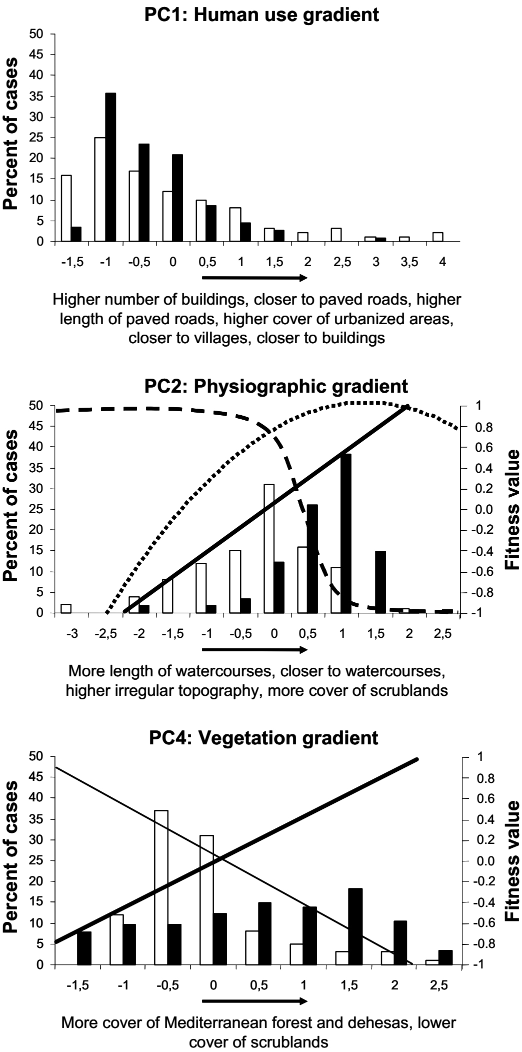

Fig. 1. Proportion of random (unfilled bars) and nest sites (filled bars) along factor scores obtained for habitat preference gradients. Variables noted bellow the abscissa are listed in order of decreasing importance in PCA and are given only if they had loadings > 0.4. The association between frequency of clutch loss (solid and broad line), clutch size (solid and narrow line), chick residual body mass (punted line) and chick heterophil/lymphocyte ratio (dashed line) along habitat gradients is represented. No association between number of fledglings and habitat gradients was found. For illustrative purposes, predicted values from regression equations for each fitness component have been standardized.

Table 2. Logistic regression (binomial error and logit link function) analyzing the probability of finding an Eagle Owl nest along three habitat gradients.

	Estimate	±S.E.	df	χ^2	*P*
Intercept	0.269	0.224			
PC1*PC1 (human use gradient)	–0.447	0.166	1, 215	7.2	0.007
PC2 (physiographic gradient)	2.194	0.305	1, 215	51.6	<0.001
PC2*PC2	0.329	0.076	1, 215	19.0	<0.001
PC4 (vegetation gradient)	1.008	0.206	1, 215	23.9	<0.001

3.2. Habitat preferences

The logistic regression analysis showed that the chance of finding an Eagle Owl nest increased along the physiographic (non-linear trend) and vegetation gradient and decreased with the human use gradient (non-linear trend; Table 2; Fig. 1). However, I found no statistically significant difference between occupied and random points along the third component ($\chi^2 = 0.91$, $P = 0.341$). Thus, nests are located in areas with high irregular topography index, length of watercourses and cover of scrublands as well as close to watercourses. Otherwise, Eagle Owls preferred nesting in areas covered with low altered natural vegetation formations like Mediterranean forest and dehesas and avoided areas showing high degree of human use (Table 2; Fig. 1).

3.3. Consequences of habitat preferences

Probability of clutch loss was negatively correlated with nest position along the physiographic gradient (Table 3; Fig. 1), suggesting directional selection for this trait. Clutch size increased and then decreased along the physiographic gradient (Table 3; Fig. 1). Furthermore, clutch size increased with NND. Number of fledged chicks was not associated with any of the studied variables (Table 3; Fig. 1). Both the vegetation and physiographic gradient and NND showed a positive relationship with residual body mass (Table 3; Fig. 1). Heterophil/lymphocyte ratio decreased with nest location along the vegetation gradient (Table 3; Fig. 1), indicating that Eagle Owls settled in preferred habitats raise higher quality offspring. As is well established for several bird species, clutch size and fledgling quality (measured by the residual body mass and heterophil/lymphocyte ratio) declined with the timing of breeding (Perrins 1970). The stability of Eagle Owl territories was higher in preferred positions along the physiographic and vegetation gradients and there was a weak evidence for a negative quadratic association with the vegetation gradient (Table 4). Territory stability showed a weak negative association with the percentage of years that the pair lost the clutch before the territory was abandoned (estimate ± S.E. = –0.025±0.013, $\chi^2 = 4.2$, $P = 0.058$, $n = 15$). The occurrence of rabbits in the diet of Eagle Owls decreased along the human use gradient, increased along the physiographic gradient and showed a negative quadratic pattern along the vegetation gradient (Table 5).

4. Discussion

In comparison with random points, Eagle Owl nests were preferentially located in areas with a high index of irregular topography, cover of scrubland, length of and proximity to watercourses (physiographic gradient). Also, Eagle Owls chose high cover of Mediterranean forest and dehesas (vegetation gradient) and areas with low human use (human use gradient). Both this and previous patterns of non-random occupation of habitat observed in Eagle Owls (e.g. Penteriani *et al.* 2001, Martínez *et al.* 2003, Ortego & Díaz 2004, Sergio *et al.* 2004) suggest a long-term process of natural selection on habitat preferences (Clark & Shutler 1999).

I found evidence of ongoing natural selection on nest-site habitat preferences in Eagle Owls, suggesting that they were adaptive mainly in terms

Table 3. GLMMs analyses for frequency of clutch loss (binomial error and logit link function), clutch size and number of fledglings (Poisson error and log link function) and fledgling residual body mass and heterophil/lymphocyte ratio (normal error and identity link function). Three habitat preference gradients, their quadratic terms and nearest neighbor distance were initially included as explanatory variables in all models. Julian laying date was included as a predictor in all models except for clutch loss analysis. Brood size was included as covariate in the analyses of residual body mass and heterophil/lymphocyte ratio. Territory identity (all models) and year (only in models for clutch loss, clutch size and number of fledglings) were included as random factors.

	Estimate	±S.E.	Test	*P*
Clutch loss				
Intercept	1.398	2.375		
PC2 (physiographic gradient)	–5.228	1.310	$F_{1,34.2} = 15.9$	<0.001
Territory identity	36.992	8.981	$Z = 4.1$	<0.001
Year	21.913	15.503	$Z = 1.4$	0.08
Clutch size				
Intercept	0.318	0.287		
PC2 (physiographic gradient)	0.579	0.203	$F_{1, 21.3} = 8.2$	0.009
PC2*PC2	–0.308	0.109	$F_{1, 21.2} = 8.1$	0.01
Nearest neighbor distance	0.272	0.085	$F_{1,20.1} = 10.3$	0.004
Julian laying date	–0.002	0.001	$F_{1, 4.21} = 8.6$	0.04
Territory identity	0.027	0.009	$Z = 2.8$	0.002
Year	0.003	0.003	$Z = 1.0$	0.15
Number of fledglings				
Intercept	0.998	0.036		
Territory identity	0.020	0.013	$Z = 1.6$	0.06
Year	0.001	0.003	$Z = 0.4$	0.33
Residual body mass				
Intercept	–290.430	158.810		
PC2 (physiographic gradient)	61.634	34.367	$F_{1,22.9} = 3.2$	0.09
PC4 (vegetation gradient)	46.509	15.896	$F_{1,22.2} = 8.6$	0.008
Nearest neighbor distance	156.36	52.301	$F_{1,21.4} = 8.9$	0.007
Julian laying date	–4.576	0.719	$F_{1,24} = 40.5$	<0.001
Nest identity	3097.800	1423.010	$Z = 2.2$	0.02
Heterophil/lymphocyte ratio				
Intercept	0.785	0.110		
PC4 (vegetation gradient)	–0.087	0.043	$F_{1,80} = 4.1$	0.05
Julian laying date	0.005	0.002	$F_{1,80} = 5.6$	0.02
Nest identity	0	0	–	–

of probability of clutch loss and offspring performance. The probability of clutch loss seemed to be subjected to directional selection along the physiographic gradient. Although clutch loss could be associated with nest desertion in some cases, depredation is the most likely cause of reproductive failure during the incubation stage (Martin 1998). By settling in inaccessible areas Eagle Owls could minimize the chance of nest detection and subsequent clutch predation, strongly suggesting that natural and/or "human" predators are an important selective agent shaping habitat preferences in Eagle Owls. This pattern could be particularly clear in the study area due to the cliff scarcity and the very high owl population density which takes many pairs to occupy places which could be considered as marginal in other populations (Ortego & Díaz 2004). Given that clutch loss is the greatest cause of reproductive failure in the study population (J. Ortego, unpublished data), occupying preferred positions along the physiographic gradient probably imposes the most important fitness benefit in relation with habitat choices.

Clutch size increased and then decreased along the physiographic gradient but this pattern was not translated to the number of fledged chicks. This indicates that the lower clutch size in pairs located in most and least preferred positions along the phys-

Table 4. Logistic regression (binomial error and logit link function) analyzing the stability of Eagle Owls territories. Three habitat preference gradients, their quadratic terms and nearest neighbor distance were included as explanatory variables.

	Estimate	±S.E.	df	χ^2	*P*
Intercept	1.382	0.369			
PC2 (physiographic gradient)	0.842	0.359	1,115	5.5	0.019
PC4 (vegetation gradient)	0.482	0.219	1,115	4.8	0.028
PC4*PC4	–0.340	0.198	1,115	2.9	0.086

Table 5. GLMM analysis with binomial error and logit link function for the occurrence of rabbits in the diet of Eagle Owls. Three habitat preference gradients and their quadratic terms were included as explanatory variables. Territory identity and year were included as random factors.

	Estimate	±S.E.	Test	*P*
Intercept	2.940	0.781		
PC1 (human use gradient)	–1.600	0.735	$F_{1,31} = 4.7$	0.04
PC2 (physiographic gradient)	3.086	0.801	$F_{1,30} = 14.8$	<0.001
PC4 (vegetation gradient)	1.012	0.495	$F_{1,29} = 4.2$	0.05
PC4*PC4	–1.115	0.437	$F_{1,30.5} = 6.5$	0.02
Territory identity	7.404	2.458	$Z = 3.0$	0.001
Year	0.0003	0.002	$Z = 0.1$	0.44

iographic gradient is probably compensated by differential effects of unmeasured parameters such as hatching success or brood size reduction. Thus, the observed association between clutch size and the physiographic gradient, which suggests stabilizing selection for this trait, is not likely to have relevant consequences on fitness. On the other hand, both the physiographic and the vegetation gradients were positively correlated with fledgling residual mass and the last one was also weakly associated with the heterophil/lymphocyte ratio, suggesting that these fitness components are subjected to directional selection. Fledgling quality is known to be positively associated with offspring survival prospects after fledgling in several bird species (Ydenberg *et al*. 1995) and, thus, could contribute greatly to the lifetime parent fitness, leading to long-term selection for pairs occupying preferred habitats.

I found partial evidence that increased chick quality in preferred habitats could be associated with better foraging opportunities. In spite of not directly measuring abundance of rabbits, the main local prey, there is evidence that preferred habitats show higher wild rabbit availability: 1) it is well established that wild rabbits select areas linked with watercourses and Mediterranean vegetation patches, where they can find soft soils to dig warrens, high protection cover and adequate food supply (Villafuerte *et al.* 1995, Virgós *et al.* 2003); 2) the occurrence of rabbits in the diet of Eagle Owls, an estimate of local rabbit availability (Serrano 2000), mostly increased in preferred habitats. Further, preferred habitats could also offer open habitats suitable for hunting rabbits such as valley floors and dehesas that may increase the probability of prey capture. Thus, by settling in preferred habitats Eagle Owls probably obtain benefits associated with better foraging performance. However, contrary to a previous study (Serrano 2002), I did not find any direct effect of the occurrence of rabbits in the Eagle Owl diet on breeding performance (data not shown). This result could be a consequence of the rough approximation taken here to estimate rabbit availability and more detailed studies may reveal the expected effects of rabbit availability on the species breeding performance.

The absence of association between habitat preference gradients and number of fledged chicks may be linked with the very high rabbit abundance in the study area (Villafuerte *et al.* 1995) which results in the highest reproductive outputs reported for Eagle Owls to date (Ortego 2004). Thus, in contrast to other European populations experiencing poorer trophic conditions (Penteriani *et al.* 2002, Marchesi *et al.* 2002, Penteriani *et al.* 2004, Sergio *et al.* 2004), pairs which start the reproduction and do not suffer nest failure (e.g. by predation) would be equally able to raise the same number of fledglings as a consequence of having superabundant trophic resources. However, the saturation threshold of food supply for fledgling condition shows a higher value than the saturation threshold for the number of fledged chicks (Tremblay *et al.* 2003) and the observed effects of habitat preference gradients on fledgling quality suggest that such a threshold has not been reached in the study population.

As found in previous studies (Marchesi *et al.* 2002; but see Penteriani *et al.* 2004), a high local owl population density reduced certain components of Eagle Owl breeding performance, including clutch size and fledgling quality. Higher population densities are likely to increase the competition for food, the rates of food depletion and the energetic and time costs related with territory defense, factors which are expected to cause a reduction in the reproductive performance (Lõhmus & Vali 2004).

Finally, I found that territory stability was mostly higher in preferred habitats, providing partial evidence of adaptive response to spatial variation in fitness in the Eagle Owl study population. This could respond to directional selection against adults settled in non-preferred habitats (e.g. by higher mortality rates) or, alternatively, the obtained pattern may represent differences in nest-site fidelity (Clark & Shutler 1999). Breeding dispersal towards better areas following reproductive failure is common in birds (e.g. Clark & Shutler 1999) and I have certain evidence that this could occur in the study population: Eagle Owls settled in non-preferred habitats along the physiographic component showed higher frequencies of clutch loss and reproductive failure tended to negatively influence territory stability. Another possibility is that pairs settled in non-preferred habitats have dispersed towards areas with better foraging opportunities (Sergio *et al.* 2003). Unfortunately, no data on territory establishment are currently available and future studies should be focused on testing the expected density increase in better habitats and analyze temporal changes of habitat preferences (e.g. Sergio *et al.* 2003).

Overall, the Eagle Owl habitat selection process in the study area seems to be mainly driven by a direct search for adequate nesting points and, in to a lesser extent, for suitable foraging areas. By selecting protected nest-sites Eagle Owls reduce the chance of clutch predation whereas preferences for habitat clues linked with better foraging performance probably affect offspring quality. These results suggest that optimal habitats correspond with the most preferred ones so that management strategies favoring their conservation are likely to be the most adequate policy to preserve the study Eagle Owl population. Furthermore, additional conservation benefits can be reached by protecting habitats preferred by Eagle Owls, given that the species seems to be associated with less-altered habitats and has been recently identified as a biodiversity indicator (Sergio *et al.* 2004).

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Habitaatin vaikutuksia huuhkajan lisääntymismenestykseen ja reviirien säilyvyyteen

Huuhkajat suosivat alueita, joiden pinnanmuodot olivat epäsäännöllisiä sekä pusikkoisia tai välimerellisen kasviston peittämiä. Ihmisasutusta huuhkajat välttelivät. Suosituimmilla alueilla pesineiden huuhkajien pesinnät epäonnistuivat harvemmin kuin toissijaisilla alueilla pesineiden yksilöiden pesinnät. Tämä johtui luultavasti pienemmästä pesäpredaatiopaineesta. Suosituilla alueilla pesineet parit tuottivat parempikuntoisia poikasia kuin toissijaisilla alueilla pesineet. Poikasten pesyekohtaisessa lukumäärässä alueiden välillä ei ollut eroja. Ravinnonkäytön seurannan perusteella on luultavaa, että erot poikasten kunnossa johtuvat alueellisista eroista ravinnon laadussa. Toissijaisilla pesimäalueilla sijainneet reviirit eivät olleet yhtä pitkäaikaisia kuin suosituilla alueilla olleet. Tämä saattaa johtua suuremmasta kuolleisuudesta tai suuremmasta muuttopaineesta toissijaisilta pesimäalueilta. Tutkimus tähdentää suojelualueiden laadun merkitystä tehtäessä luonnonsuojelullisia päätöksiä huuhkajan osalta.

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Appendix. Environmental variables measured at Eagle Owl nests and random points. The land use types provided by Corine Land Cover maps grouped into ten categories are indicated.

Variable	Description
Human use related variables	
Distance to village (m)	Distance to the nearest village or town
Number of buildings	Number of buildings within the circular area sampled
Distance to building (m)	Distance to the nearest building
Distance to road (m)	Distance to the nearest paved road
Distance to unpaved road (m)	Distance to the nearest unpaved road
Length of paved roads (m)	Meters of paved roads within the circular area sampled
Density of unpaved roads	Number of unpaved roads crossed by four axes (N–S, W–E, NW–SE and NE–SW) from the plot centre
Land use variables	
Non-irrigated herbaceous crops (%)	Sum of the covers of: 1) Non-irrigated arable land; and 2) Land occupied mainly by agricultural uses with some areas of natural vegetation
Irrigated crops (%)	Sum of the covers of: 1) Permanently irrigated lands; and 2) Other irrigated lands
Perennial crops (%)	Sum of the covers of: 1) Vineyards; 2) Olive groves; 3) Mixtures of perennial crops; 4) Irrigated orchards; and 5) Mixtures of annual and perennial crops
Tree plantations (%)	Sum of the covers of: 1) Coniferous forest; and 2) Other broad-leaved tree plantations
Dehesa (%)	Cover of: 1) Agro-forestry areas
Pasture (%)	Sum of the covers of: 1) Other pastures; and 2) Saline
Scrubland (%)	Sum of the covers of: 1) Low-density scrub and scrubland; and 2) High scrubland formations of medium to high density
Mediterranean forest (%)	Sum of the covers of: 1) Forests of evergreen sclerophyllous and Lusitanian oaks; and 2) Transitional woodland-scrubland
Water (%)	Sum of the covers of: 1) Rivers and natural water courses; and 2) Reservoirs
Urbanized areas (%)	Sum of the covers of: 1) Discontinuous urban fabric; 2) Continuous urban fabric; 3) Green urban areas; 4) Industrial or commercial units; 5) Mineral extraction sites
Ecotone number	Number of habitat boundaries crossed by two axes (N–S and W–E) from the plot centre
Physiographical variables	
Irregular topographic index	Number of contour lines crossed by two axes (N–S and W–E) from the plot centre
Distance to watercourse (m)	Distance to the nearest valley floor
Length of watercourses (m)	Meters of valley floors within the circular area sampled