SEX-RATIOS OF AN ENDANGERED LARK AFTER CONTROLLING FOR A MALE-BIASED SAMPLING

RAZÓN DE SEXOS EN UNA ALONDRA AMENAZADA DESPUÉS DE CONTROLAR EL SESGO DE CAPTURAS HACIA LOS MACHOS

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Avian sex ratios, their deviation from the expected by random genotypic sex determination, and the direction of the skew are considered of great biological and conservation significance, in particular for species with reduced populations (see a recent review in Donald, 2007). One case of particular concern among European avifauna is the Dupont's lark (*Chersophilus duponti*), a passerine species restricted to the iberian peninsula where occurs in small populations scattered among different regions, with a total population estimated during the last spanish census around 3,500 - 4,200 territorial males (years 2004 - 2007; Suárez and Garza, in press; see also Garza *et al*., 2003; 2004; Tella *et al*., 2005). The species is actually considered 'Near-Threatened' by IUCN criteria (IUCN, 2008) and 'Endangered' in Spain (Garza *et al.*, 2004). Furthermore, the iberian nuclei are genetically isolated from the remainder african populations (Garcia *et al*., 2008a). As in many other passerine species (Bibby *et al*., 2000), censuses and survey data on Dupont's lark are based on counts of territorial males (e.g. García *et al*., 2008b), with the risk of overestimate the effective population size if the adult sex ratio is skewed towards males. Therefore, a better understanding of adult sex ratios is urgently needed to clarify the conservation status of this endangered species.

In regard to adult sex ratios, there is only data from one Dupont's lark population (Tella *et al*., 2004; Vögeli *et al.*, 2007) that give values of 0.79 (males outnumbered females by around 280 %), and far less is known about patterns of seasonal variation nor on sex ratios of chicks (secondary sex ratios). We here analysed adult sex ratios in two northeastern Spanish Dupont's lark populations: Alfés, Lérida (7º 14' E, 41º 25' N), and Ablitas, Navarra (5º 32' E, 41º 46' N), and the secondary sex ratio in one population in Central Spain (Layna, Soria: 5º 0' E, 40º 54' N). Data from the Alfés population was collected during 1985-91, from a population now considered extinct, whereas data from the Ablitas population were collected during 2005 - 07 from a population estimated at a minimum of 40 males (Tella *et*

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al., 2005; authors' unpublished data). In both populations, birds were captured in clap-traps placed randomly within the study areas and baited with mealworms (*Tenebrio molitor*), avoiding the use of song recordings in order to reduce sex-biases in captures. Trapping took place all year around, with an approximate sampling effort of 25 traps per day over a surface of 2 ha. The location of this 2 ha plot was different each day of capture. Captured birds were ringed to avoid possible replications. The total number of birds captured was 33 and 46 (Alfés and Ablitas, respectively). Individuals were sex assigned by means of a discriminant function based on wing length, as proposed by Vögeli *et al*. (2007). In order to explore seasonal patterns in sex-ratio, individuals were separated into two groups according to capture date: those from the breeding season (early February to late July) and non-breeders (early August to February). Finally, to obtain secondary sex ratio data, a total of 16 chicks from five different nests found at Layna in 2007 were sexed by molecular means according to the protocol described by Griffiths *et al.* (1998).

Since the capture method may bias sex ratios obtained for adult birds (Domenech and Senar, 1998; Vanderkist *et al.,* 1999), and the possible incidence of this effect in larks is unknown, we calibrated our data by well-studied behavioural parameters in this species. As other passerines, Dupont's lark shows clear between-sex differences in spacing behaviour during breeding, being the extent of male's territories about twice as large as those of females (Garza *et al.,* 2005), and a more intense patrolling behaviour is expected in males than in females, using the space in different ways (Garza *et al*., 2005). Therefore, when traps were placed randomly within the study areas, the chance to capture a male might be overall higher than the chance to capture a female, and the probability of capturing individuals in different parts of the territories might differ also between males and females. It may therefore be expected that given equal numbers of both sexes into the population, the capture rates during the breeding season may be different for males and females. In other words, departure from an expected 1:1 sex ratios based on capture data do not necessarily imply unbalanced sex ratios into the population during the breeding season. To test this hypothesis, we designed a model simulating the capturing protocol, which allowed us to construct the null hypothesis of capturability of males and females under a hypothetical population with equal number of males and females. We used the ArcGIS version 9.2 (Environmental Systems Research Institute, 2006) package, incorporating field data on territory size, shape and overlap of two breeding pairs at Layna population, which were radio-tracked between April and June 2003 (Garza *et al*., 2005). Using 13 and 45 locations for females, and 27 and 74 for males, kernel isolines were determined for each of the four individuals by means of the 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 probabilities. This method is proved robust to a potential effect of the number of locations (Worton, 1995).

The value of kernel isopleths increases outwards along the territories, but the probability of bird's presence within the rings delimited by two contiguous isopleths, increases inwards. Therefore, the probability of presence within each ring was defined as the inverse of the value of its external isopleth. Then, a grid of 2 ha-square was superposed upon this layer, and 25 random points were generated in each square. The probability of presence of each individual was then calculated for each point. The method was replicated 100 times, and points out of the 90 % isopleth received the null value.

Finally, the capture probability and its variance for each sex were calculated as the mean values of the 100 trials. The relative inter-sex capturability value obtained was applied to correct the sex ratios of birds captured during the breeding season in the two populations studied. Finally, the statistical significance of departure from an expected 1:1 sex ratio was

TABLE 1

Original and corrected by capturability values of the sex ratios obtained at Alfés (Lérida), Ablitas (Navarra) and in total for the two localities, and at the Ebro Valley according to Vögeli *et al.* (2007). The χ^2 statistic is included to determine deviations from a 1:1 ratio. Values providing differences ($P < 0.05$) are in italics.
[Valores brutos y corregidos mediante el modelo de capturabilidad de la razón de sexos obtenidos en Alfés (Lérida), Ablitas (Navarra) y en el total de las dos localidades, así como en el valle del Ebro según Vögeli et al. *(2007). Se incluye el estadístico de la Chi-cuadrado para determinar su desviación frente a la relación 1:1. Los valores con P < 0,05 se señalan en cursiva.]*

Data	Locality	Period	Males	Females	Ratio (males/total)	χ^2	P
Original	Alfés	Breeding	14	7	0.67		
		Non-breeding	6	6	0.50		
	Ablitas	Breeding	17	5	0.77		
		Non-breeding	16	8	0.67		
	Alfés and Ablitas	Breeding	31	12	0.72	4.413	0.0357
		Non-breeding	22	14	0.61	0.900	0.3428
Corrected	Alfés and Ablitas	Breeding	26	17	0.60	0.952	0.3291
	Alfés and Ablitas	Breeding and non-breeding	48	31	0.61	1.851	0.174
	Ebro Valley	Breeding and non-breeding	34	9	0.79	7.784	0.0053
	Pooled data	Breeding and non-breeding	82	40	0.67	7.450	0.0063

assessed by means of Chi-squared tests, pooling data from the two studied populations since both of them could be considered as belonging to the meta-population of the Ebro Valley (Laiolo and Tella, 2007), as belong the smaller data set obtained by Vögeli *et al.* (2007). Differences in capturability between males and females were tested by one-way ANOVA test.

Probability of capture during the breeding season differed significantly between males and females (one-way ANOVA, F = 9195.0, 1 d.f., *P* < 0.0001), being female 67.9 % of male capturability. Adult sex ratios was skewed towards males in the two populations studied, although the skew magnitude was not statistically different from the 1:1 ratio for each of the two periods considered and after correction by capturability (table 1). In both localities, the sex ratios were more skewed to-

wards males during the breeding than non-breeding period, although non significant differences were found. The secondary sex ratio observed in Layna population in five different nests was 0.50. The sex ratio described by Vögeli *et al.* (2007; table 1) did not differ significantly from those calculated for our populations (χ^2 = 4.119 and 1.222, d.f. = 2, *P* = 0.126 and *P* = 0.543 for the non-breeding and breeding periods, respectively), and differed significantly from the 1:1 ratio (table 1). Pooling our corrected data with those corrected of Vögeli *et al.* (2007) the sex ratio also differed from the 1:1 ratio (table 1).

Our model revealed that, even when apparently random capture methods were used, the probability of capture during the breeding season was sex-dependent (female being 0.67 of male capturability), probably due to in-

ter-sex differences in the use of space. After the sex ratios obtained from captures during the breeding season were corrected for the relative capturability of each sex, their values became similar to those from the non-breeding period and did not differ statistically from the 1:1 ratio, except when considering our data together with the dataset obtained from Vögeli *et al.* (2007). However, the biological interpretation of our findings should be taken cautiously. The sample size required to prove a bias in sex ratio of 0.60 ($\alpha = 0.05$) with a good statistical power (i.e. 0.80) is 118 individuals. Here we have analysed less than 80 individuals, which suggest results are likely but prevent us to reach undoubted statistical support. Other studies using data-simulation procedures, nevertheless, found estimates of proportions (exemplified by parasite prevalences) that comply with real data even with sample sizes as low as 15 individuals (Jovani and Tella, 2006). Our results suggest a slight male-biased sex ratio in the studied populations, and help to explain the presence of floating males during the breeding period in some populations (Garza *et al.*, 2005; Vögeli *et al.* 2008). Although the sex ratio determined by Vögeli *et al.* (2007) in the Ebro Valley population is difficult to compare with our data (since the authors do not distinguish captures between periods), some bias in sex ratio towards males fit well also with their data.

Overall, the obtained value in this study (mean value 0.61 during both breeding, after correction by capturability of each sex, and non-breeding periods), is the second most frequent value among the 201 bird species reviewed by Donald (2007). The secondary sex ratio observed in Layna gives equal number of female and male chicks in five sampled nests, in agreement with data recovered in other avian species (Donald, 2007).

Our correction factor, however, should be applied cautiously due to the following reasons: (i) it assumes that energy requirements and the extent of corresponding foraging dis-

placements remain constant throughout the breeding season for both sexes; and (ii) due to the lack of similar studies, it is based on parameters calculated from territories of a different Dupont's lark meta-population, which could involve some between-site differences in the relative size of male and female territories during breeding period. Therefore, it is likely to assume differences in the relative capturability of each sex, even when "random" capture methods were used, and we consider necessary to account for this source of error on sex ratios studies of this and other species.

The high rates of nestling mortality observed in this species (Herranz *et al.*, 1994) suggest an overall greater female mortality, as seems to occur widely in birds (Sillett and Holmes, 2002; Liker and Székely, 2005; Githiru and Lens, 2006). Another possible cause of this discrepancy between sexes is that females disperse more widely, either by the proportion of individuals which move or by the average distance travelled, which could bring about higher female mortality (Clarke *et al.*, 2002; Dale, 2001; Steifetten and Dale, 2006). The later possibility, however, is less likely to occur due to the reduced distance of dispersion registered in Dupont's Lark (Laiolo *et al.*, 2007). Moreover, the probability of receiving immigrants is markedly low in small and isolated populations (Laiolo and Tella, 2008), as in the case of Alfés and Ablitas.

These findings hold major implications for the conservation of Dupont's lark. A tertiary sex ratio of 0.61 mean that in a population with 100 registered males only contain 65 females and thus, potential breeding pairs. Under such assumptions, the maximum effective population size in Europe will be ca. 2,700 breeding pairs, which is a very small population size for a passerine. Indeed, many sub-populations actually hold less than 10 males, being highly exposed to the extinction due to the reduced number of effective breeding pairs (Laiolo *et al.*, 2008; Suárez and Garza, in press). Further studies in other regions and accounting for variable

population size are needed to study in depth the potential effect of bias in sex-ratio for the conservation of the species.

RESUMEN.—*Se estudia la razón de sexos de los adultos de alondra ricotí* (Chersophilus duponti) *en dos localidades del valle del Ebro, y de los pollos en una localidad del Sistema Ibérico (Layna, Soria). Los pollos (n = 16) se sexaron molecularmente, resultando una razón de sexos de 0,50. Los adultos se capturaron mediante cepos dispuestos al azar y sin reclamo y fueron sexados biométricamente, resultando una razón de sexos de 0,72 durante el periodo reproductor y 0,61 fuera de éste. La capturabilidad teórica de cada sexo durante el periodo de reproducción, analizada mediante un modelo que considera las características de los territorios de machos y hembras, fue un 32 % menor en las hembras. Al corregir la razón de sexos obtenida durante la época reproductora mediante el resultado de este modelo, la razón de sexos fue de 0,61, valor idéntico al obtenido durante el periodo no reproductor y no diferente estadísticamente de la relación teórica 1:1. No obstante, esta razón de sexos ligeramente sesgada hacia machos hace disminuir el número de parejas efectivas de la población europea de la especie, incrementando su grado de amenaza.*

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