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Owl dusk chorus is related to individual and nest site quality

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Dawn and dusk choruses represent one of the most investigated topics in bird vocal behaviour, but their underlying basis remains unclear. Dusk chorus in owl seems to support the mate and rival assessment hypothesis (like the dawn chorus in passerines) and happens during the most constraining period as individuals have not fed yet and, under the handicap principle, dusk chorus is likely to reveal inter-individual differences in e.g., competitive ability, body condition and/or habitat quality. Here, the study of vocal displays at dusk of 14 Eagle Owls *Bubo bubo* revealed a temporal succession in the order in which males began their vocalizations. The vocalization order appears to be related to: (i) the quality of the nesting territory (i.e., mean number of fledged young and amount of rats in the diet); and (ii) the male's quality, as revealed by haematocrit values and the brightness of the white throat patch, a signal of individual quality.

Key-words: *Bubo bubo*, calls, ~~dusk chorus~~, individual quality, vocal display

Bird dawn and dusk choruses (the sunrise and sunset peaks in song output), represent one of the most investigated topics in vocal behaviour (Bradbury & Vehrencamp 2011). During choruses: (i) various individuals are involved in interactive communication (Hardouin *et al.* 2008, Foote *et al.* 2010, 2011); (ii) the frequency of interactions depends on the relationships among males (Foote *et al.* 2008); ~~and~~ (iii) the timing of vocal displays varies among individuals (Foote *et al.* 2011) and may be related to male quality (Otter *et al.* 1997, Murphy *et al.* 2008). However, after more than two decades of study, the basis underlying twilight choruses remains unclear (Staicer *et al.* 1996, Burt & Vehrencamp 2005, Bradbury & Vehrencamp 2011) and studies have primarily focused on diurnal songbirds (but see Hardouin *et al.* 2008, Penteriani & Delgado 2009).

Eagle Owls *Bubo bubo* perform dawn and dusk choruses (Delgado & Penteriani 2007), the dusk chorus being analogous to the dawn chorus of diurnal birds. When studying the dusk chorus of neighbouring males we detected a phenomenon never ~~before~~ reported before in avian choruses, i.e. a temporal succession in the order in which individuals start their vocalizations. Here, we characterise such a pattern and identify which factors may determine this order. Our main objective ~~is~~ was to understand whether individuals: (1) living in high quality sites and/or (2) in better condition call earlier/for longer than individuals in low quality sites and/or of low quality, respectively; ~~and~~ (3) breeding on slopes exposed to sunlight for ~~a shorter~~ less time ~~call~~ perform vocal displays at higher rates than those in sunnier places.

METHODS

Vocal behaviour

During 67 listening sessions, we documented the vocalizations of 14 breeding males (mean distance between neighbouring breeding sites: $\bar{x} \pm \text{SD} = 1007 \pm 480$ m, min = 250 m, max = 1760 m) of a population in south-western Spain (Sierra Morena). Listening sessions were conducted during 2011 and 2012, in the pre-laying period (September - January), the annual vocal activity peak of Eagle Owls (Delgado & Penteriani 2007). Vocal behaviour was monitored from 1 hr before to half an hour after sunset (~~which warranted the to~~ guarantee observation of the first owl vocalizations), one of the two daily peaks of Eagle Owl calls (Delgado & Penteriani 2007). During listening sessions we recorded: (i) time of the first and all subsequent calls; and (ii) vocal display duration (minutes) ~~of vocal bouts (series of single male hoots)~~. We defined the end of a bout as the last call heard >60 sec before the next call (Penteriani 2002). We did not conduct observations on windy or rainy days because of ~~the~~ interference with call displays and call detection by observers.

We arranged the 14 individuals into 4 neighbouring groups (two groups of three and two groups of four neighbours; sampling effort per group: group 1 = 22 nights; group 2 = 17; group 3 = 17; group 4 = 11). To avoid subjectivity when clustering the focus males, Groups-groups were formed on the basis of: (i) ~~the proximity of individuals~~: distances among the closest individuals of the same group ($\bar{x} \pm \text{SD} = 964 \pm 186$ m) were shorter than distances among the closest individuals of neighbouring groups ($\bar{x} \pm \text{SD} = 1170 \pm 309$ m); and (ii) ~~the~~ location of suitable listening places from where it was possible to listen to the individuals of each ~~sub~~group. The clustered made using proximity criteria and the location of suitable places for recording is mostly coincident with other methods of spatial segregation (Hernández & Navarro 2007).

To test if the order in vocal displays differs from chance, we created a null model of emergence time to ~~compare which the~~with real data ~~could be compared~~. ~~This was accomplished by~~To do this, for all 67 nights, we randomly ~~sampling~~sampled one male from each of the four ~~neighbourhoods~~groups repeatedly and ~~calculating~~calculated the % that each male would have started first. ~~This was repeated for all 67 nights to create the null data set.~~

Finally, we calculated (Harper 1994): (i) repeatability of call order, which provides an additional indication of behavioural stability; ~~and~~ (ii) repeatability of time to sunset of the first vocalization. This may help determine whether call order is a by-product of something internal (a fixed time to sunset, ~~which may be~~possibly based on individual quality independent of other birds) or external (interactions with neighbouring owls).

Breeding site quality and exposure

Quality of breeding sites was estimated by: (a) mean egg laying date and mean number of fledglings; (b) availability of rabbits *Oryctolagus cuniculus*, the main prey of the population (mean biomass percentage in diet = 62.0 ± 19.1 %), within the nest site, estimated by the number of latrines per km of transects within an area equal to the mean Eagle Owl home range size in our population (more details in Campioni *et al.* 2013); ~~and~~ (c) the % ~~of~~ biomass of rabbits and rats in the diet. Rats represent the second most common species in the Eagle Owl diet and increase nesting site quality (Campioni *et al.* 2013).

Nest site exposure is a categorical variable composed of 8 classes, the main cardinal points, and corresponds to the amount of light illuminating the vicinity in which males started vocalizations. This represents the possible effect of ambient light in triggering the commencement of vocal displays.

~~The~~iIndividual quality

The physiological condition of ~~the~~ 10 males that were trapped (out of ~~the~~ 14 ~~total~~) was estimated by (Campioni *et al.* 2013): (a) haematocrit (HT), an indicator of nutritional status; and (b) body condition index (BCI, a reduced major axis regression using log of body mass and wing length; Delgado *et al.* 2010), with the highest values corresponding to the best individuals. We also measured (c) the brightness (i.e., total reflectance) of the white feather patch on the male throat, which is positively correlated with individual quality (Bettega *et al.* 2013).

Statistical analyses

To explain the frequency (%) each individual began the dusk chorus within ~~the~~ a group of ~~neighbours~~ (dependent variable), we ran three models related to the quality of the breeding site, the exposure of the nest site and individual quality. First, we conducted a graphical analysis for the explanatory variables and checked for Spearman's rank correlations among predictors, excluding variables with $r_s \geq 0.6$. The frequency each individual began the dusk chorus was log-transformed and the response variables were normalised to zero mean and unit variance. Backward selection of variables was applied to the full model, and models were compared using likelihood ratio tests (Crawley 2007). Residuals of the final models were explored to verify the assumptions of normality and independence. Statistical analyses were performed using R 2.10.1 statistical software (R Development Core Team 2009).

RESULTS AND DISCUSSION

The call starting time ranged from 56 to 1 minute before sunset ($\bar{x} \pm SD = 22.8 \pm 9.9$ min). The earliest males to stop vocalizations did so 2 min after sunset ($\bar{x} \pm SD = 18.6 \pm 3.5$ min). Mean time of starting calls before sunset was different among the groups (from 1 to 4: 41, 17, 30 and 31 min; $\chi^2 = 9.77$, $df = 3$, $P = 0.02$), whereas average ending time of calls after sunset was homogeneous (from 1 to 4: 18, 22, 20 and 26 min; $\chi^2 = 1.63$, $df = 3$, $P = 0.65$). After the first individual of a group started calling, the second one did not always begin vocalizing immediately ~~after~~ ($\bar{x} \pm SD = 21.0 \pm 4.2$ min after the first individual started to call; min = 1 min; max = 69 min); ~~total~~ Total duration of vocal displays ranged from 37 to 370 min ($\bar{x} \pm SD = 145.6 \pm 88.6$ min).

Order in vocal displays

Dusk choruses were initiated by the same individual of each neighbouring group in most cases. For groups 1 to 4, the same individual started first in 93.8%, 52.9%, 50.0% and 36.4% of choruses, respectively, and in three out of the four groups there ~~were one or two~~ was at least one individuals that never called first. The order of vocal displays was different from the null model, where the same individual for groups 1 to 4 started first in 34.6%, 28.2%, 35.9% and 30.0% of the simulated choruses, respectively; for the null model, the commencement of vocalizations was never started by the same individual. An ordered call sequence was also evident for the individuals that occupied the second (53.9%, 66.7%, 40% and 36.4% of the cases) and third (45.5%, 50.0%, 64.7% and 42.9%) ranking in their respective groups. Repeatability for call order (0.80) was higher than that for time to sunset (0.52), suggesting that, independent of sunset, individual features appeared to play an important role in choruses.

~~The owl choruses thus determined the emergence of a hierarchical communication based on the characteristics of individual quality and the quality of the nest territory. The~~

~~nights in which~~ When the 'usual early' caller was not first, the overall order did not change radically, as the 'usually second' caller became ~~the~~ first (the third and fourth individuals did not change their order). The habitual order typically changed because the usually first owl started to vocalize later.

Nesting site and individual attributes help explain chorus hierarchy

Males exhibiting the highest fecundity and proportion of rats in their diet (adjusted R-squared = 0.33, $F_{2,25} = 7.71$, $P = 0.003$; Table 1A), as well as the highest HT and brightness (adjusted R-squared = 0.82, $F_{2,7} = 20.96$, $P = 0.001$; Table 1B) were the ones which started call ~~displays~~ earliest. Nest site exposure (adjusted R-squared = -0.07, $F_{2,25} = 0.15$, $P = 0.86$) did not affect hierarchy. The owl choruses thus determined the emergence of a hierarchical communication based on the characteristics of individual quality and the quality of the nest territory.

~~Results~~ Our results support the contention that calls can carry information related to both the individual's state and habitat quality ~~features of the place that individuals inhabit.~~

The information contained in calls may be considered an honest signal of individual and territory quality (Penteriani *et al.* 2002, Hutchinson *et al.* 1993, Hoi-Leitner *et al.* 1995): similar to that suggested by Montgomerie (1985) for the dawn chorus, energy reserves of nocturnal species should be lowest at sunset and may impose a handicap in the timing and amount of vocalizations. Experimental evidence supports the idea that birds in food-rich territories show higher call rates than individuals in poorer territories (Hoi-Leitner *et al.* 1995) and, more generally, that high display rates are associated with physiological costs (e.g., Oberweger & Goller 2001), which only individuals in better (body) condition ~~better conditioned individuals~~ are able to afford (Hardouin *et al.* 2007). The ability to maintain a high song output may reflect the ability of the male to acquire the resources needed to

allow time for early/longer vocal displays. This efficient resource acquisition is probably mediated by the occupation of high-quality territories (Reid 1987, Hutchinson *et al.* 1993).

A quality-mediated chorus might support the assertion that vocal displays arbitrate social relationships with territorial neighbours through interactive communication, as proposed by the social dynamics hypothesis (i.e., males sing to adjust their relationships with neighbours; Staicer *et al.* 1996, Foote *et al.* 2008), and also hypothesized for Little Owls *Athene noctua* (Hardouin *et al.* 2008).

The dusk chorus can thus emerge as a result of multiple external stimuli, such as sunset (Penteriani & Delgado 2009), a high density of conspecifics and signals from early callers, where focal individuals orchestrate a sort of behavioural synchrony within the communication network.

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Table 1. Final linear regression models showing the effect of (A) breeding site quality and (B) individual features on Eagle Owl call order

	Estimate	SE	<i>t</i>	<i>P</i>
Log (call order)				
A				
Intercept	2.24	0.28	8.04	<0.0001
% rat biomass	0.82	0.29	2.88	0.008
Mean fecundity	0.68	0.29	2.39	0.025
B				
Intercept	27.02	3.03	8.91	<0.0001
Hematocrit	16.92	3.33	5.07	0.001
Badge brightness	17.67	3.33	5.30	0.001