Experimentally increased prebreeding male social behaviour has no effect on female breeding phenology and performance

Allison Cornell[*], James J. Hou, Tony D. Williams

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

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In the context of breeding phenology, social behaviour (especially song) has long been considered an important 'supplemental cue' that females integrate with other environmental information to determine timing of egg laying. However, to our knowledge, no studies have experimentally manipulated song in the wild in the context of female breeding phenology and performance. We studied natural variation in, and response to experimental manipulation of, prebreeding song and social behaviour in European starlings, *Sturnus vulgaris*, to determine whether male behaviour acts as a phenological cue determining female timing of egg laying and subsequent, postlaying breeding performance. In our highly synchronous system, natural variation in prebreeding male song quality and singing effort was surprisingly high, and singing increased slightly closer to egg-laying date. There was a strong prebreeding response of both males and females to the playback treatment, but no effect on female breeding phenology (egg-laying date) or performance (nestbox occupancy, egg size, clutch size, total chicks hatched, total chicks fledged or double-brooding behaviour). Our 3-year study finds no evidence that male prebreeding song is a cue determining female breeding phenology or performance in starlings.

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Males use vocalizations, such as songs or calls (Catchpole & Slater, 2003; Davidson & Wilkinson, 2004; Rand & Ryan, 1981; Searcy & Andersson, 1986; Vannoni & McElligott, 2008), and behavioural displays (Andersson & Iwasa, 1996; Clark & Morjan, 2001) to communicate with conspecifics during the breeding season. These male signals can be important for attracting a mate, defending a breeding territory and protecting female mates and offspring from other males (Adkins-Regan, 2005; Catchpole & Slater, 2003; Davidson & Wilkinson, 2004; de Kort, Eldermire, Cramer, & Vehrencamp, 2009; Rand & Ryan, 1981). While females have been shown to prefer males that exhibit exaggerated traits, such as more complex song (Eens, 1997; Gentner & Hulse, 2000; Lauay, Gerlach, Adkins-Regan, & DeVoogd, 2004; but see Byers & Kroodsma, 2009; Rand & Ryan, 1981) or more time spent singing (Houtman, 1992), less is known about the role of male social behaviours after mate selection occurs. However, the production of sexually selected traits such as song can be costly (Walther & Clayton, 2005) and therefore should inform female breeding decisions (Harris & Uller, 2009). In birds, male prebreeding song has

been studied extensively, but rarely in the context of natural variation in breeding phenology in free-living birds, which is a primary determinant of lifetime reproductive success (Daan, Dijkstra, & Tinbergen, 1990; Dawson & Clark, 2000; Lepage, Gauthier, & Menu, 2000; Verboven & Visser, 1998). Instead, birdsong has primarily been investigated in the context of mate choice (Byers & Kroodsma, 2009; Houtman, 1992; Lauay et al., 2004; Searcy & Yasukawa, 1996), territory defence (Illes, Hall, & Vehrencamp, 2006; de Kort et al., 2009) and neural development (reviewed in: Beecher & Brenowitz, 2005; Brenowitz & Beecher, 2005). Furthermore, when social cues are examined in the context of breeding phenology, the emphasis is often on the effects on conspecific males rather than egg-laying females (Dufty & Wingfield, 1986; Moore, 1983; Tramontin, Wingfield, & Brenowitz, 1999; but see Cheng, Desiderio, Havens, & Johnson, 1988), and few studies extend beyond the prebreeding period and examine effects of male social behaviour and song on female breeding phenology or performance, especially in the field.

In the context of breeding phenology, social behaviour (especially song) has long been considered an important 'supplemental cue' that females integrate with other environmental information to determine their timing of egg laying. In breeding birds, photoperiod is the primary environmental cue that predicts a window of time for reproduction, but within this window even slight

* Correspondence: A. Cornell, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
E-mail address: acornell@sfu.ca (A. Cornell).

variation in the timing of egg laying can have major fitness consequences (Daan et al., 1990; Dawson & Clark, 2000; Lepage et al., 2000; Verboven & Visser, 1998). The supplemental cues that then 'fine-tune' laying date within the wider photoperiodic window, have long been assumed to include temperature (e.g. in 'cold' versus 'warm' springs), food availability and social cues (Wingfield, 1983; Wingfield & Farner, 1993). However, although correlational studies between timing cues and reproductive behaviour have shown links to temperature (Dunn, 2004; Schaper et al., 2012), food availability (Van Noordwijk, McCleery, & Perrins, 1995), leafing of plants (Bourgault, Thomas, Perret, & Blondel, 2010) and social interactions (Wright & Cuthill, 1992), experimental studies have largely been unsuccessful in confirming which of these cues is the main determinant of individual variation in egg-laying date, especially in females (Caro, Schaper, Hut, Ball, & Visser, 2013; Williams, 2012). In relation to social cues, laboratory studies have shown that male presence can increase female body mass and ovarian follicle development under appropriate photoperiod conditions (e.g. Cheng et al., 1988; Stevenson et al., 2008). However, most studies that have reported effects of song on female reproductive physiology and behaviour have been conducted in the laboratory, so they did not measure egg-laying date or subsequent female reproduction (Hinde & Steele, 1976, 1978; Kroodsma, 1976; MacDougall-Shackleton, MacDougall-Shackleton, & Hahn, 2001; Morton, Pereya, & Baptista, 1985). In outdoor aviaries female European starlings, *Sturnus vulgaris*, will undergo full vitellogenesis and follicle maturation, and Perfito, Guardado, Williams, and Bentley (2014) showed that final maturation of the female reproductive system is sensitive to social interactions: restricting male access completely inhibited final follicle maturation, whereas 7-day male access stimulated full vitellogenesis and follicle maturation in females. In one of the few relevant field studies, Wright and Cuthill (1992) found a positive correlation between earlier laying dates and male singing time in European starlings, but this study was nonexperimental. To our knowledge, the only experimental field study that used song playback treatment during prebreeding and that considered any effects on female reproduction showed an increase in nest-building behaviour, but it only examined prelaying effects (i.e. not effects of egg-laying dates or subsequent female breeding performance; Mota & Depraz, 2004).

Here we studied natural variation in male prebreeding song and social behaviour in European starlings and then experimentally manipulated the level of social stimulation using song playback. Our specific focus was to determine whether male behaviour during the prebreeding period acts as a phenological cue determining female timing of egg laying and subsequent postlaying female breeding performance. This study took place in a free-living population with highly synchronous egg laying (see Williams et al., 2015) where social cues are potentially more likely to regulate breeding (Smith, 2004). Our objectives were specifically to (1) identify how natural variation in singing effort and song quality change throughout the prebreeding period (1 month prior to egg laying, cf. Wright & Cuthill, 1992), (2) determine whether natural variation in these metrics of singing effort and song quality relate to nestbox occupancy, egg-laying date, clutch size, egg mass, number of chicks hatched, number of chicks fledged and double-brooding behaviour, (3) measure male and female behavioural responses to an experimental increase of song during prebreeding, and (4) most importantly, to determine whether experimentally elevated levels of male song and social behaviour affect breeding phenology (egg-laying date) and reproduction (nestbox occupancy, clutch size, egg mass, number of chicks hatched, number of chicks fledged and double-brooding behaviour) in females.

METHODS

Natural Variation in Prebreeding Song and Behaviour

We collected 350 song recordings during prebreeding nestbox activity beginning approximately 30 min after dawn, during 4 March–10 April 2014 at Davistead Farm, Langley, British Columbia, Canada (49°10′12″N, 122°49′48″W). Birds at each of the 75 nestboxes studied were recorded approximately once per week, four to six times in total. Voice recorders (Olympus VN-722PC and Olympus VN-702PC) were placed underneath the nestboxes in plastic bags attached with thumbtacks. Recording response frequency range was 70 Hz to 16 kHz and playback range was 20 Hz–20 kHz. Bags were left under the boxes on nonrecording days to accustom starlings to their presence. Each recording was analysed for the level of singing effort (percentage of time spent singing, number of bouts/h) as well as song quality (mean bout length, mean number of motifs/bout). We used the definitions of song bouts (a period of singing for at least 5 s with pauses no more than 1.5 s) and song motifs (distinct repeated song phrases) from Eens (1997). Bout length and number of motifs are strongly correlated, and bout length has been linked to attractiveness to females in starlings (Gentner & Hulse, 2000). We analysed 55/350 recordings for the entire recording length (2 + h), and for the remaining recordings, we analysed 10 singing bouts, starting with the first 30 min of the recording (there was no significant difference between the subsample of 10 bouts and the entire recording sample; paired t test: mean bout length: $t_{1,54} = -1.5$, $P = 0.15$; mean motifs: $t_{1,54} = -1.6$, $P = 0.11$). The same observer analysed all 350 recordings using Raven Lite (Bioacoustics Research Program, 2016). We calculated the percentage of time spent singing by taking the sum of all bout lengths and dividing by the recording time (either whole recording or total subset length analysed) × 100. Song rate, or number of bouts per hour, was the number of singing bouts (regardless of length) observed per hour of total observation time. We calculated mean bout length by averaging bout length in seconds for each nestbox and averaging the mean number of motifs per bout. Because visual surveys (see below) were not conducted during the entire +2 h recording period, we cannot verify with certainty that all songs on each recording were always of the same male; only that recordings were made in the same territory (i.e. at the nestbox). Therefore, we refer to these results by discussing male song in the territory or at a nestbox.

Simultaneous visual observations of both male and female prebreeding behaviour were made 4 days/week for all nests ($N = 75$ nests) during the song recording period. We followed the 10 min survey methods described in Wright and Cuthill (1992), although in our study each observer only covered one to two boxes per observation period. Boxes were observed in random order, and thus any time of day or rainfall effects were randomized in the data set. The data collected from these surveys included the percentage of time males were present at the nestbox, percentage of time males were singing at the nestbox, number of male and female entries to the nestbox (which we use as a proxy for nest-building activity) and other social interactions (e.g. aggression, copulation). Rainfall was recorded during surveys categorically as 'heavy rain', 'medium rain', 'light rain', or 'clear' (since we knew a priori that rainfall could influence singing behaviour).

Experimental Manipulation of Prebreeding Song

In 2015 and 2016 we experimentally manipulated prebreeding social cues using daily playbacks of 'high-quality' European starling song starting on 14 March (13 days prior to egg laying in 2015, 19 days prior to egg laying in 2016) until 1–2 days after the first egg