Female canaries invest more in response to an exaggerated male trait

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Females choose their mate on the basis of secondary sexual characters such as physical ornaments, courtship behaviours or vocalizations. In male birds, vocalizations are known to increase their attractiveness and consequently their probability of copulation. Moreover, male songs have been shown to shape maternal investment, particularly with regard to egg quality. Artificial manipulation of male attractiveness has been found to affect female sexual preferences and maternal investment. In this regard, supranormal stimuli are thought to increase such responses. The present study was designed to assess whether supranormal acoustic stimuli, known to induce high levels of sexual responsiveness in female canaries, Serinus canaria, could increase maternal investment beyond levels observed in birds exposed to natural songs. Exposure to supranormal songs (G phrases) increased maternal investment: compared to females exposed to moderately attractive (Control) or naturally attractive (A) phrases. Females exposed to supranormal songs laid bigger eggs (egg volume and mass, albumen mass); moreover, females in the G group laid eggs with heavier yolks than females in the Control group. In contrast, no differences were found between groups with regard to testosterone deposition in the eggs. Thus, the adjustment of females' investment in their brood seems to take various forms that appear to be related to different features of males' sexually selected traits, which raises the questions of how and why these different mechanisms have been selected.

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According to theories of honest signalling such as Zahavi's 'handicap principle', females select mates on the basis of sexual ornaments and signals that honestly reveal a male's quality (Zahavi 1975, 1977). These indicators of male quality are considered by females during mate choice and can differ according to the species. For instance, some species use body size or coloration while others use vocalizations (Andersson 1994; Bortolotti et al. 2006). In songbirds, male quality is thought to be related to the characteristics of songs (Gil & Gahr 2002). Indeed, parameters such as singing activity, repertoire size, type or intensity of vocalizations can provide information about male quality. For instance, in swamp sparrows, Melospiza georgiana, females display more in response to songs with a frequency bandwidth and trill rate that come near to the limits of vocal performances in this species (Ballentine et al. 2004). In swamp sparrows, the ability to perform physically challenging songs predicts both the age and size of males (Ballentine 2009).

The influence of these honest signals is not limited to mate choice: females also use them to adjust their reproductive effort (e.g. Cunningham & Russell 2000; Horváthová et al. 2012). The hypothesis of adjusting parental investment in response to the traits of partners, when they represent honest signals of quality, is known as the differential allocation hypothesis (Burley 1988). Using zebra finches, Taeniopygia guttata, Burley showed that individuals allocate more parental care to offspring born of better-quality mates (Burley 1986, 1988). An artificial alteration of males' sexually selected characters in field or laboratory experiments can induce dramatic changes in female investment. Kingma et al. (2009) artificially masked the UV coloration of the crown feathers of male blue tits, Cyanistes caeruleus. Then, they evaluated the reproductive investment of females by measuring the levels of androgens deposited in the eggs. The authors found that a reduction in the UV reflectance in males' feathers caused a rapid decrease in testosterone concentrations in the eggs. This example demonstrates that partial erasing of male sexually selected traits weakens maternal investment of their mate. In the same way, in the blue-footed booby, Sula nebouxii, several studies showed that females decreased the size of their eggs when the feet of their mates were made duller (Velando et al. 2006; Dentressangle et al. 2008); such a rapid adjustment of the investment in the egg in response to

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changes in the male's sexual traits was seen as adaptive. Conversely one might expect that artificial enhancement of male traits with supranormal stimuli would positively affect female reproductive activity. This seems to be the case: Andersson (1982) showed that long-tailed widowbird, *Euplectes progne*, males whose tail was experimentally elongated showed higher mating success than males having a normal or reduced tail. A positive effect of supranormal stimuli on female preferences was also found in a few other experiments (Sullivan 1983; Rowland 1989; and see Christy 1995 for a theoretical approach of courtship signals' mimicry). However, if the positive influence of supranormal male characters on female sexual responsiveness seems to be established, the question of whether supranormal stimuli can increase maternal investment more than natural ones remains open.

To answer this question, we used domestic canaries, *Serinus canaria*. In this songbird, both female sexual preferences and maternal investment are correlated with male song characteristics. For instance, females listening to playbacks of large song repertoires laid larger clutches than females listening to playbacks with smaller repertoires (Kroodsma 1976). In addition, females show a high sexual responsiveness to special phrases called 'A' or 'sexy' phrases (Vallet & Kreutzer 1995). These A phrases influence female preferences but also female reproductive investment: indeed, females produced larger eggs when they were exposed to sexy songs rather than to nonsexy songs (Leitner et al. 2006; but see Gil et al. 2004; Tanvez et al. 2004). Finally, females exposed to the diffusion of natural attractive phrases laid eggs with higher amounts of testosterone than females exposed to unattractive songs or without any acoustical stimulation (Gil et al. 1999; Tanvez et al. 2004; but see Marshall et al. 2005). While female domestic canaries use male songs as indicators of male quality, males also exhibit interesting features. They sing phrases composed of two-note syllables. Depending on both the bandwidth they cover and the repetition rate at which they are produced, these syllables will be more or less attractive for females. Attractive syllables that cover a large bandwidth and are produced at a high rate require particular skills to coordinate the two parts of the syrinx (Suthers et al. 2004, 2012; Vallet et al. 2006). In addition, there is a trade-off between the frequency bandwidth and the production rate: a high display rate implies a smaller frequency bandwidth and vice versa (Podos 1997; Suthers et al. 2012). Thus, these song parameters seem to be an honest signal from males. Attractive A phrases (that cover a large frequency bandwidth of 4 kHz and are produced at a rate of 16 syllables/s) seem to be at the limit of the canary species' capacity (Podos 1997; Suthers et al. 2012); previous studies did not find natural songs exceeding these parameters (Draganoiu et al. 2002). In addition, in the aforementioned experiment, Draganoiu et al. (2002) broadcast phrases exceeding this limit. To build these 'G' phrases, they took two-note syllables covering a 6 kHz frequency bandwidth that are normally produced at tempos lying between 5 and 14 syllables/s. By artificially increasing the production rate to 16 or 20 syllables/s, it seems that they created supranormal stimuli; females were very responsive to these 'G' phrases (Draganoiu et al. 2002). However, these authors did not investigate the effect of these G phrases on maternal effort. Overall, the domestic canary is a good biological model to answer the question of whether supranormal stimuli can increase maternal investment more than natural ones as (1) females adjust their sexual preferences and investment to male songs, (2) these songs seem to be honest signals and (3) G phrases seem to be supranormal stimuli.

To answer this question, we exposed laying females to three types of phrases: (1) F phrases covering a 2 kHz bandwidth; (2) A phrases covering a 4 kHz bandwidth; and (3) G phrases covering a 6 kHz bandwidth. All these phrases were made of two-note syllables produced at a rate of 16 syllables/s. F phrases were our control songs as they are moderately attractive and are broadly produced by most of the males during the reproductive season; A phrases were normal attractive songs and G phrases were supranormal songs.

We hypothesized that supranormal G songs would increase maternal investment more than natural sexy (A phrases) or control songs (F phrases) in terms of size of the clutch, weight and size of the eggs and testosterone concentrations in the yolk as suggested by previous studies that investigated the influence of natural stimuli on maternal investment (Kroodsma 1976; Gil et al. 1999; Tanvez et al. 2004; Leitner et al. 2006).

METHODS

Study Animals

The subjects of this study were 38 2–6-year-old female canaries with previous reproductive experience that we randomly selected from the breeding stock of the laboratory (LECC Université Paris Ouest, Nanterre-La Défense, France). Water and food (Beyers seed mix, containing all necessary nutrients for breeding females, and an egg-based mix, Cédé) were provided ad libitum. Before, during and after the experiments, the birds were kept in rooms in controlled conditions (22 ± 1 °C).

Before the experiment, females were housed in single-sex breeding cages (38 × 33 cm and 26 cm high, six birds per cage) on a short-daylight schedule (8:16 h light:dark). Females were allowed to hear males singing in adjacent cages in the same room.

One week before the experiment, females were removed from the breeding cages, randomly assigned to an experimental group (see below) and housed in individual cages (38 × 33 cm and 26 cm high) in an experimental room. Cages were placed in sound attenuation chambers (90 × 50 cm and 80 cm high inside) so that females were exposed to the experimental songs only and did not hear any other male song during the experiment. To avoid social isolation, two females were placed in the same chamber and thus both females heard the same song sequences.

Each cage was provided with a piece of cuttlebone for calcium; each female was also given a nest bowl and nesting material (cotton strings, Sharpie Benelux nv). The nesting material was renewed every day. Females were checked daily for nest building and egg laying throughout the experiment.

In the experimental room, the photoperiod was on long days (16:8 h light dark) to induce reproductive activity (Follett et al. 1973). During the experiment, we broadcast the experimental songs to females twice a day for 2 h each time. The song bouts were composed of 15 song sequences each 2 min long (see Fig. 1 for details of songs) separated by silences. Each song sequence contained six song repetitions. Overall, females heard 90 song repetitions.

Females were assigned to one of the three experimental groups: 10 females were exposed to F songs (Control group), 16 females to A songs (A group) and 12 females to G songs (G group). Age did not differ significantly between groups (ANOVA: $F_{2,37} = 0.208$, $P = 0.813$).

Experimental Songs

All the songs used during this experiment were computer-edited songs. We took complex syllables from a collection of natural domestic canary songs previously recorded in the LECC with a Marantz PMD 201 tape recorder. Songs were built using the Avisoft-SasLab software, v.3.74 (Avisoft Bioacoustics, Berlin, Germany). Tested phrases were inserted between introductory notes and concluding notes to obtain 6 s long songs (Fig. 1). These phrases