Genes, environments and their interaction: song and mate choice in canaries

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ARTICLE INFO

Male secondary sexual traits and female mate choice traits must contain heritable variation for sexual selection to operate. However, for female mate choice, especially, this is poorly known. To complicate matters, both male sexual traits and female mate choice typically show condition dependence, implying that environmental effects probably play an important synergistic role. Using a cross-fostering design, we therefore aimed to disentangle genetic, environmental and their potential interacting effects to investigate how they affect the expression of a sexually selected trait (here birdsong) and female mate choice. To assess environmental effects, we focused on the role of the social environment and thus on learned components. Among the different male song traits investigated, we found a high heritability for song bout length and song bout repertoire, as well as an intriguing gene-by-environment interaction for song bout repertoire. Specifically, the tutor appeared to negatively affect the song bout repertoire of the tutee when his genetic father had a large song bout repertoire but had a positive effect on descendants from fathers with small song bout repertoires. In contrast, we did not detect significant heritability in female mate choice. Female mate choice contained a learned component as females significantly disfavoured their foster father in the mate choice tests, indicating a learned inbreeding avoidance. Thus, our study provides important insights into the role of the social environment for both birdsong and female mate choice. However, the absence of a heritable component in female mate choice suggests a need for studies investigating the heritability of female preferences for (heritable) male song traits in order to gain a better understanding of a potential coevolution between male sexual traits and female mate choice. Overall, our results suggest that indirect genetic effects acting during the pre- and postfledging social contexts may play a prominent role in sexual selection.
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Sexual selection is characterized by intraspecific reproductive competition between individuals of one sex to get access to gametes of the other sex. It acts on those traits that enhance reproductive success, either through increased chances of success in competition for mates (intrasexual selection) or via traits that make them more attractive for the opposite sex (intersexual selection; reviewed by Kokko, Jennions, & Brooks, 2006; Hosken & House, 2011). The latter is typically achieved via secondary sexual characteristics that might be reliable signals of quality and hence affect mate choice (Forstmeier, Burger, Temnow, & Derégnaucourt, 2009; Holman, 2012; Zahavi, 1975). Intersexual selection may ultimately give rise to coevolution via reciprocal selective effects between signalling traits, typically in males, and the degree of preference for it by the other sex, typically the females (Kokko et al., 2006;

Qvarnström, Brommer, & Gustafsson, 2006; Ryan & Kirkpatrick, 1991).

Selection requires that traits, here male sexual traits and female mate choice, are heritable (Ryan & Kirkpatrick, 1991). Heritability of sexually selected male traits has indeed been shown in a number of previous studies (e.g. Evans & Sheldon, 2012; Forstmeier et al., 2009; Hadfield & Owens, 2006; Hubbard, Jenkins, & Safran, 2015; Karino & Haijima, 2001; Müller, Vergauwen, & Eens, 2010). However, as reflected in the often low heritability estimates, especially for behavioural traits, sexually selected traits contain not only a genetic but also a significant environmental component.

Birdsong for example, a well-studied sexually selected trait that honestly signals male quality for female mate choice (Eens, Pinxten, & Verheyen, 1991) and plays a role in male–male competition (Williams, 2004), is in fact a largely learned behaviour (Nottebohm, Kasparian, & Pandazis, 1981). Male song characteristics therefore depend on the male's genetic predisposition to learn, and thus on specific brain regions that are involved in song control, which have a strong genetic component (Airey, Castillo-Juarez, Casella, Pollak,

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& DeVoogd, 2000; Garamszegi & Eens, 2004; but see also ; Leitner & Catchpole, 2004; Woodgate et al., 2014). However, what can be learned will depend on the exposure to other individuals' songs during a critical period in early life (Bolhuis & Gahr, 2006; Nowicki, Peters, & Podos, 1998; Williams, 2004), that is their social environment. In fact, it is likely that male song will depend on an individual's capacity to learn as well as the quality of the song templates to which it is exposed, reflecting gene-by-environment interactions (GxE). Empirical studies have shown that GxE effects are widespread for male sexual traits (e.g. David, Fowler, Bjorksten, & Pomiankowski, 2000; Lewandowski & Boughman, 2008; Rodriguez & Al-Wathiqui, 2011), including song expression, here investigated in the context of nutritional stress (Woodgate et al., 2014). However, such synergistic effects of genes and environments have received little attention in the context of song learning.

In turn, female mate choice is also an important fitness-related trait because females gain direct and indirect benefits by mating with high-quality males (Kodric-Brown & Brown, 1987; Ryan & Kirkpatrick, 1991), but our knowledge of its heritability is even more limited. Several studies, in a large range of taxa, have found significant heritability of choice-related parameters, such as the time the females invest in assessing the males or how selective the females are (Brooks & Endler, 2001; Brooks, 2002; Rodríguez & Greenfield, 2003). However, a general pattern on the heritability of preference functions (i.e. the actual outcome of female mate choice) is currently lacking despite its impact on phenotypic trait evolution via intersexual selection (Prokuda & Roff, 2014; Schielzeth, Bolund, & Forstmeier, 2009; Zietsch, Verweij, Heath, & Martin, 2011). Moreover, repeatability estimates of these preference functions are generally low in birds (Bell, Hankison, & Laskowski, 2009; Forstmeier & Birkhead, 2004), while repeatabilities are often considered as upper bounds for heritability. As mentioned above, low heritability estimates indicate a relatively large influence of the environment, both abiotic (e.g. Holveck & Riebel, 2010; Woodgate, Bennett, Leitner, Catchpole, & Buchanan, 2010) and biotic, given the evidence for social learning (Bolhuis & Honey, 1998; Riebel, 2000; ten Cate, 2006; Ludwig & Becker, 2008; but see; Hegyi et al., 2010). As in males, G×E effects may be very common, but studies that have investigated G×E effects for female mate choice are remarkably scarce (Ingleby, Hunt, & Hosken, 2010; 2013).

In this study, we aimed to partition phenotypic variance in both male sexual traits and female mate choice traits into their genetic and their environmental variance components, as well as to investigate potential G×E effects. To this end, we manipulated the social environment of male domesticated canaries, *Serinus canaria*, to investigate three different sources of variation in male song: (1) genetic effects (via son–genetic father comparisons); (2) early environmental effects (via son–foster father comparisons); and (3) later environmental effects during the song-learning period (via son–tutor comparisons). As song represents a multifaceted signalling behaviour (Gil & Gahr, 2002), we quantified four different song parameters in male canaries (i.e. song activity, average bout duration, song bout repertoire and song consistency) that have previously been shown to be important in the context of sexual selection (see for instance: Kroodsma, 1976; Searcy, 1992; Eens et al., 1991; Gil & Gahr, 2002; Botero, de Kort, Eldermire, Valderrama, & Vehrencamp, 2009; Müller et al., 2010). In a similar and simultaneous approach, we assessed the contribution of genetic (via daughter–genetic mother comparisons) and learned early environmental effects (via daughter–foster mother comparisons and by testing the effect of male familiarity on female mate choice) to the outcome of both female mate choice and choice-related parameters. All birds experienced similar and constant husbandry conditions throughout their lives, which enabled us to

specifically focus on the contribution of genetic background and social environment to the expression of our assessed traits.

METHODS

Study Species and Animal Husbandry

We used a total of 157 Fife Fancy canaries, originating from our own laboratory stock population with a known pedigree since 2006. More precisely, the songs of 44 males (hatched in 2011 or 2012) were recorded in 2014, including 38 genetic fathers (F0 generation), 36 foster fathers and 17 tutors. The songs of 51 young males, which hatched in 2014 and which descended from the 38 genetic fathers (F1 generation, subsequently referred to as 'sons'), were recorded in 2015. The mate choice experiment was performed in 2015, with 31 mothers (F0, hatched in 2011 or 2012) and their 31 genetic daughters (F1).

The F1 generation was cross-fostered as nestlings at a very early stage (day 0 = hatching), with all foster nests containing four unrelated nestlings to standardize parental workload across nests (for more details, see Iserbyt, Eens, & Müller, 2015). At cross-fostering, all nestlings of a foster nest were of similar age (maximum 12 h difference between nestlings) to equalize competitive abilities among nestlings. Furthermore, foster nestlings hatched from eggs with a different egg order to minimize inflation of maternal effects within nests (Schwabl, 1993). Each foster family was housed separately (GEHU cages measuring 50 × 64 cm and 40 cm high). All birds experienced a long light regime (14:10 h light:dark) throughout this period and had access to canary seeds (van Camp, Boechout, Belgium) and water ad libitum. They received enriched egg food (van Camp, Belgium) daily, as well as germinated seeds.

The second phase of the experiment started when the F1 generation reached 25 days. This period corresponds with nestling independence, when young canaries no longer rely on provisioning by the parents. After 25 days, all birds from the F1 generation were subdivided into groups of eight individuals together with an adult tutor male. Tutor males were chosen randomly from our outbred laboratory population. Tutor groups were housed in larger separate cages (50 × 128 cm and 40 cm high) in visual and semiacoustic isolation. The light regime was switched to a short light regime (10:14 h light:dark). Young females were removed from tutor groups after molecular sex determination (Griffiths, Double, Orr, & Dawson, 1998) from a 100 μl blood sample taken on day 25. All F1 females were housed along with their mothers in large aviaries separated from the males. All birds stayed in these conditions for approximately 1 year until 5 weeks prior to the experiments in February 2015. At this time, the light regime was switched to long light (14:10 h light:dark) to stimulate their reproductive activities.

Ethical Note

Our research was approved by the Ethical Committee of the University of Antwerp (ID: 2014–72). Our study involved video and song recordings to monitor natural animal behaviour (see below) and was for this reason free from pain or distress. Blood sampling did not compromise the wellbeing of the birds, given that natural behaviour (e.g. brooding or singing) was resumed usually within 15 min following manipulation. Long-lasting effects of these manipulations were never observed in our study population.

Birdsong

For song recordings, all males (F0 [tutors, genetic and foster fathers] in 2014 and F1 sons in 2015) were housed individually in separate cages (GEHU cages 50 × 64 cm and 40 cm high), starting