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Variation in song system anatomy and androgen levels does not correspond to song characteristics in a tropical songbird

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Variation in song structure and song production of birds are thought to relate to variation of both androgen levels and neural nuclei in the song system, as typically these nuclei are larger in males than in females, vary in size among males and are sensitive to steroid hormones. We investigated the relationships among song and note structure, singing rate, androgen levels and the sizes of two song nuclei, the higher vocal centre (HVC) and the robust nucleus of the arcopallium (RA) in male and female red-backed fairy-wrens, *Malurus melanocephalus*. Males of this duetting species express three discrete reproductive phenotypes that differ in plumage colour and behaviour. Although HVC and RA structure differed between the sexes, there were no sex differences in note structure and complexity of songs, although females differed from some male types in song rate and frequency characteristics. Both auxiliary males and females had significantly lower androgen levels than the two breeding male phenotypes. Male reproductive phenotypes had similar song characteristics and HVC and RA structure, but differed in androgen levels. Sexes and male phenotypes varied in song rate, but these differences did not correspond to differences in androgen levels. Thus, sex differences in song nuclei anatomy and androgen levels were not associated with differences in song structure and singing rate; and, the differences in androgen levels among male phenotypes were not reflected in differences in singing rate, song structure or the song nuclei. We conclude that, similar to other recent findings, the sexes of the red-backed fairy-wren can produce similar song and express similar singing behaviour despite differences in song system structure and circulating androgen levels; singing and song system anatomy appear not to be part of the suite of traits associated with differences in androgen levels in male red-backed fairy-wrens.

Avian song and the neural song system is a key model system for studies of behavioural and neural sex differences, neuroplasticity and brain–behaviour relationships, and therefore offers unique opportunities to unravel the roles of genes, hormones and the social environment in moulding brain and behaviour. The song system is a network of forebrain nuclei that functions in learning and production of song in songbirds. In this network, the nuclei higher vocal centre (HVC) and robust nucleus of the arcopallium (RA) comprise the motor pathway to muscles of the syrinx that, via medullary nuclei, control the production of song, particularly the repertoire of note types and syllable types within songs (Bolhuis & Gahr, 2006; Nottebohm & Arnold, 1976).

HVC and RA are typically larger in male songbirds than in female songbirds, but the degree of size dimorphism varies substantially among species (MacDougall-Shackleton & Ball, 1999: results from 20 species). In species in which song is restricted to males, such as the zebra finch, *Taeniopygia guttata*, HVC and RA are much larger in males than in females, whereas in species in which both sexes sing and produce songs of similar structure, sexual size dimorphism of both nuclei is reduced (Brenowitz, 1997; Brenowitz & Arnold, 1986; Gahr, 2007; MacDougall-Shackleton & Ball, 1999). These observations have led to the hypothesis that sex differences in neural space determine sex differences in structure and complexity of motor output (Nottebohm, Kasparian, & Pandazis, 1981). However, sexual

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dimorphism of song nuclei volume also occurs in species where the sexes have similar song production and sing similar or near similar song (i.e. Brenowitz, 1997; Brenowitz & Arnold, 1986; Gahr & Metzdorf, 1997; Gahr, Sonnenschein, & Wickler, 1998; Hall, MacDougall-Shackleton, Osorio-Beristain, & Murphy, 2010; Jawor & MacDougall-Shackleton, 2008; Voigt & Gahr, 2011).

Sizes of the song nuclei also vary in individuals of the same sex, probably due to the influence of environmental and hormonal factors. In males of seasonally breeding species the sizes of HVC and RA vary with reproductive stage (e.g. Nottebohm et al., 1981), photoperiod (e.g. Tramontin, Hartman, & Brenowitz, 2000) and androgen levels (e.g. Small, Brenowitz, & Moore, 2007). Social context also influences male singing and song system anatomy (Sartor & Ball, 2005; Sockman, Salvante, Racke, Campbell, & Whitman, 2009; Strand, Ross, Weiss, & Deviche, 2008), and the volumes of male song nuclei vary with social status in species with complex social organization of breeding units. For example, dominant males of the white-browed sparrow weaver, *Plocepasser mahali*, have larger HVC and RA volumes and differ from subordinate males in the cellular machinery and gene expression patterns in these nuclei (Voigt & Gahr, 2011; Voigt, Leitner, & Gahr, 2007). Variation in male song nuclei size is assumed to relate to differences in song structure and repertoire size at the individual, population and species level (Airey & DeVoogd, 2000; Canady, Kroodsma, & Nottebohm, 1984; DeVoogd, Krebs, Healy, & Purvis, 1993; Garamszegi & Eens, 2004; Kirn, Clower, Kroodsma, & DeVoogd, 1989; Leitner & Catchpole, 2004), its evolution driven by sexual selection and limited by proximate mechanisms (Gil & Gahr, 2002; Podos, Huber, & Taft, 2004).

The gonadal steroid hormone testosterone could be a common physiological denominator to explain sex differences, seasonal plasticity, social modulation and species differences in song nuclei volume, singing and song structure. First, female songbirds generally have much lower testosterone levels than males (Goymann & Wingfield, 2014; Ketterson, Nolan, & Sandell, 2005; Møller, Garamszegi, Gil, Hurtrez-Boussès, & Eens, 2005), and exogenous testosterone increases female song nuclei volumes (Brown & Bottjer, 1993; Madison, Rouse, Balthazart, & Ball, 2014; Nottebohm, 1980) and stimulates singing in females of several species (e.g. Appeltants, Ball, & Balthazart, 2003; Hausberger, Henry, & Richard, 1995; Kern & King, 1972; Kriner & Schwabl, 1991; Lahaye, Eens, Darras, & Pinxten, 2012; Madison et al., 2014; Rasika, Nottebohm, & Alvarez-Buylla, 1994; Voigt & Leitner, 2013; and references in Rosvall, 2013). Second, seasonal plasticity of singing and song structure and of song nuclei anatomy in males of photoperiodic species is, at least in part, regulated by changes in testosterone levels (e.g. Bernard & Ball, 1997; Gulledge & Deviche, 1997; Small et al., 2007; Smith, Brenowitz, & Wingfield, 1997; Tramontin, Wingfield, & Brenowitz, 2003). Third, exogenous testosterone increases song repertoire size and singing rate in seasonally nonreproductive males (Van Hout, Pinxten, Darras, & Eens, 2012) and exogenous testosterone metabolites modify song nuclei anatomy (Hall & MacDougall-Shackleton, 2012). Fourth, male social status in breeding groups is often associated with differences in androgen levels (e.g. DuVal & Goymann, 2011; Ryder, Horton, & Moore, 2011; Soares et al., 2010; Wingfield & Lewis, 1993).

We examined the relationship between singing rate, song structure, song nuclei anatomy and circulating androgen levels in male and female red-backed fairy-wrens, *Malurus melanocephalus*, a cooperatively breeding Australian songbird. In this tropical species both sexes sing, as do other members of the songbird family Maluridae (i.e. Greig, Price, & Pruett-Jones, 2013; Hall & Peters, 2008; Rowley & Russel, 1997). All group members, including auxiliary helper males, sing and all join their songs to form overlapping, polyphonic duets and choruses that appear to function in intergroup territorial interactions (Dowling & Webster, 2013). Red-backed fairy-wren songs are similar to the chatter song of the superb fairy-wren, *Malurus cyaneus* (Dalziell & Cockburn, 2008) and the type I song of the splendid fairy-wren, *Malurus splendens* (Greig & Pruett-Jones, 2008). Unlike these species, the red-backed fairy-wren sings only one song type, but varies the number, type and order of notes within songs (Dowling & Webster, n.d.-b).

Male red-backed fairy-wrens express three morphologically and behaviourally distinct reproductive phenotypes: ornamented (red/black plumage) breeders, unornamented (brown, female-like plumage) breeders, and unornamented auxiliary helpers (Karubian, 2002; Webster, Karubian, & Schwabl, 2010). Male phenotypes join mates and other group members in singing duets and choruses (Dowling & Webster, 2013), but differ in their sexual and parental behaviour (Karubian, 2002), sperm production (Rowe, Swaddle, Pruett-Jones, & Webster, 2010) and reproductive success (Webster, Varian, & Karubian, 2008). Male types vary dramatically in androgen levels: during all reproductive stages, red/black males show the highest, brown breeders intermediate and brown auxiliaries the lowest androgen levels (Lindsay, Webster, Varian, & Schwabl, 2009). The androgen testosterone (T) is both necessary and sufficient to induce the ornamented male plumage phenotype, as T levels during the prenuptial moult predict red/black versus brown breeding plumage phenotype (Lindsay et al., 2009) and T implants induce a prenuptial moult into the red/black male plumage phenotype (Lindsay, Webster, & Schwabl, 2011). Androgen levels rise when males change social status from auxiliary helper to breeder, and this transition is associated with changes in bill colour and size of the cloacal protuberance (Karubian, Lindsay, Schwabl, & Webster, 2011), both of which are androgen-regulated traits (Donham, Wingfield, Mattocks, & Farner, 1982; Laucht, Kempenaers, & Dale, 2010).

Given these behavioural and hormonal differences among male breeding types, this species is well suited to examine the relationship among androgens, brain song centres and acoustic output. The present study investigates whether singing rate, song structure and its underlying neural motor pathway (nuclei HVC and RA) are part of the suite of traits associated with variation of androgen levels in alternative reproductive phenotypes of male red-backed fairy-wrens, and whether predicted sex differences in androgen level are associated with differences in singing rate, song structure and anatomy of song control regions. Based on the hypotheses outlined above, we predicted that differences between male phenotypes in androgen levels would be reflected by differences in singing rate, song structure and anatomy of the song nuclei HVC and RA. We further predicted that lower androgen levels in females than in males would be reflected in sex differences in singing rate, song structure and song nuclei size.

METHODS

Study Species

We studied a population of colour-banded red-backed fairy-wrens near Herberton, Queensland, Australia (145°25′E, 17°23′S), which has been monitored continuously since 2003. The study sites are located in open sclerophyl forest with tall eucalypt overstory and grass understory. The reproductive biology of this nonmigratory species is described in detail by Webster et al. (2010). Breeding is seasonal, lasting from September through April (with occasional nesting thereafter), although onset varies with rainfall (Webster et al., 2010). Like other members of the Maluridae (Peters, Kingma, & Delhey, 2013), red-backed fairy-wrens moult twice per year with most birds assuming cryptic, brown plumage during the