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Clearing up the court: sex and the endocrine basis of display-court manipulation

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Sexual selection can drive the evolution of novel traits, including behaviours, that may arise in sex-specific patterns and be under sex-steroid hormone control. In some polygynous species, males actively manipulate their display environment, likely to influence female perception of male sexual traits. As a presumptive appetitive reproductive behaviour, the motivation to manipulate the display environment may be activated by reproductive hormones and the degree of motivation may vary across individuals. To evaluate these possibilities, we examined the hormone dependence of court-clearing behaviour in lek-breeding golden-collared manakins, *Manacus vitellinus*, of Panama where males clean the rainforest floor of debris within display courts and maintain these cleaned courts during the long breeding season and during subsequent years. Nonbreeding females and juvenile males were given implants containing testosterone (T) or empty (control) implants and observed in a large aviary situated in the middle of Panamanian rainforest. The aviary was planted with saplings around which males display; the bare ground was covered with dried leaves. Control-treated males removed only a few leaves from their courts. T treatment dramatically stimulated court-cleaning behaviour, including both the removal of leaves and attempts to extract small seedlings from the soil. None of the females displayed court-cleaning behaviour, although T-treated females showed low levels of some male courtship displays as well as significant levels of aggressive behaviour. To assess wild adult males' motivation to clear courts, we presented lekking males with weighted artificial leaves. Males were attentive to the leaves and motivated to remove them. With increasing weight, males utilized different methods, which often involved multiple repeated attempts to move leaves. These findings demonstrate that manakins exhibit sex-specific and hormone-dependent court-cleaning behaviours and that males are highly motivated and physically adapted to keep their courts clean.
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Sexual selection, specifically female choice, is an important driver of male display behaviour and ornamentation in many taxa (West-Eberhard, 2014). Often, signals used in attraction and courtship of females exploit multiple sensory response capacities that may have been co-opted from other contexts and elaborated upon. Complex courtship displays are particularly ubiquitous in polygynous bird species, in which males often perform acrobatic routines and possess elaborate plumages (Borgia & Keagy, 2015; Prum, 1990). The diversification of sexually selected traits has been of much interest, and behavioural innovations have been proposed to lead to or facilitate changes in morphology (Mayr, 1960; West-Eberhard, 2003). The evolution of new postures or 'moves' in some bird species are known to anticipate plumage elaborations, such as long tail feathers or bright colour patches, that enhance the performance of the behaviour (Prum, 1990).

One prevalent environmental factor that may influence signalling behaviour is the display habitat (Anciães & Prum, 2008; Endler & Thery, 1996). In many polygynous birds, males perform courtship displays at a lek within a territory and typically in the same location, such as on a particular log or branch. In several species, males also actively manipulate the surrounding environment. For example, in bowerbirds (Aves: Ptilonorhynchidae), males build exquisitely ornamented bower structures that may be assessed by females and can influence female perception of male courtship

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display (Borgia & Keagy, 2015; Endler, Gaburro, & Kelley, 2014). In Neotropical manakins (Aves: Pipridae), manipulation of the display area is most elaborate in *Manacus* species, in which males clear courts on the forest floor and trim vegetation above or surrounding the court (Chapman, 1935). Clearing the display area may have several functions, including advertising court ownership and male quality to females, but it notably emphasizes plumage ornamentation and display choreography (Coccon, Schlinger, & Fusani, 2012; Uy & Endler, 2004). In the *Manacus* species hybrid zone in Panama, reflectance from court and surrounding vegetation is hypothesized to favour the introgression of the yellow collar plumage ornament into the white collar population (Uy & Stein, 2007). In several species, including *Manacus* manakins, there can be differences among individuals in display site properties, suggesting that variation exists on which selective pressures can act, such that males choose or modify their display site to improve display quality (Anciães & Prum, 2008; Uy & Stein, 2007). In manakins, manipulation of the external environment thus represents an innovation that has appeared in several unrelated taxa and which is especially conspicuous in *Manacus* species. However, to date little is known about the mechanisms that support this behaviour and that may have contributed to its appearance. To understand what influences male responses to environmental challenges, it is necessary to examine the contribution of the underlying physiological mechanisms on the performance of court cleaning.

Here, we examine court-cleaning behaviour in the golden-collared manakin, *Manacus vitellinus*. Specifically, we address the role of androgens in the activation of court cleaning and the extent that inherent motivation and muscular strength are important in its performance. Adult males that own a court at the lek engage in cleaning daily, following practice of their courtship routine when females are not observing them (Chapman, 1935). Males maintain a court during a fairly long (>6-month) breeding season and they also often return to the same court during subsequent breeding seasons. The court is approximately 50 × 50 cm and is surrounded by small vertical saplings, which are used during the typical 'jump-snap' display. During the display, males swiftly leap between saplings while performing a wing-snap, which is produced by beating their wings together above their head in midflight, and they occasionally perform a jump-snap with a half-twist landing on the ground followed by a whirring flight back onto a sapling (a 'grunt-jump' display). The court is maintained free of debris particularly at the base of the saplings as these areas may be used for landing during the grunt-jump display.

Although court cleaning involves responses to environmental inputs, it can be viewed as an appetitive reproductive behaviour and, thus, the motivation and the neuromuscular capability to perform this behaviour may be activated differently in adults under hormonal control and/or may develop uniquely across the sexes due to developmental 'organizational' events of a hormonal or genetic basis (Adkins-Regan, 2013; Arnold, 2017; Schlinger, 1998). Previous work shows that testosterone (T) activates the motivation to perform the courtship display in juvenile golden-collared manakins (Day et al., 2007; Day, McBroom, & Schlinger, 2006). Some elements of courtship are also activated by T in females, although females perform fewer male-typical behaviours and at lower rates (Chiver & Schlinger, 2017). Here, we examined whether T treatment activates court-cleaning behaviour in both sexes. In addition, we tested male motivation and muscular strength in a court-cleaning context to determine whether males vary in their performance of this behaviour. Similar to other behaviours that involve responses to unpredictable environmental conditions, such as habitat or food choice, the development of court cleaning may involve learning or prior experience (Mayr, 1974). This would be advantageous by providing flexibility in the face of variable obstacles over time and space, and may be seen as variability among males in their motivation to explore and engage in cleaning behaviour. Muscular strength in removing heavier debris may also improve with practice and males may additionally use unique motor patterns to remove heavier debris. Here, we found that (1) court-cleaning behaviour is androgen dependent; (2) testosterone activates the behaviour in males but not in females; (3) some aspects of motivation to keep the court clean vary significantly among males; (4) males possess considerable strength and can fly off carrying heavy debris (approximately their own weight) in their beaks, which is likely an adaptation to court clearing; and (5) when challenged to remove 'heavy' leaves, males exhibit some behavioural flexibility, suggesting that prior experience may be an important factor influencing court-clearing behaviour.

METHODS

Ethical Note

The work was conducted at the Smithsonian Tropical Research Institute (STRI) in Gamboa, Panama, under permits from local Panamanian authorities (Autoridad Nacional del Ambiente and MiAmbiente permits SE/A-4-14 and SE/A-55-15) and in accordance with animal care policies at STRI (Animal Care and Use Committee Protocol number 2013-0315-2016) and at the University of California Los Angeles (Office of Animal Research Oversight, ARC Protocol number 2009-123-21), which adhere to the standards set by ASAB/ABS (2012). All birds observed in captivity were released at the point of capture following the end of experiments.

Animals and Housing

To assess effects of testosterone on court-clearing behaviour, we utilized juvenile male and female golden-collared manakins, which have basal T levels and readily respond to exogenous T treatment (Chiver & Schlinger, 2017; Day et al., 2006, 2007). Wild birds were captured near leks using passive mist netting, individually marked with plastic coloured leg bands, weighed and transferred to STRI facilities in Gamboa, where they were housed individually in small cages (~36 × 29 cm and 32 cm high). Birds were in visual and auditory contact in cages set on a table in a room with open screened windows (allowing ventilation and natural lighting). Supplemental lighting was added and timed to coincide with the ambient sunrise and sunset. Fresh papaya with vitamin supplements and water were provided twice daily (ad libitum). Once three or four birds of the same sex were available, they were assigned to either a T treatment group or a control group (see below for treatment description, Treatment Administration) and transferred to a large outdoor aviary (10 × 5 m and 4.5 m tall, with a double-door entrance 1 × 1 m and 2 m tall) located ~2 km inside Soberania National Park near the town of Gamboa. The aviary contained 45 small saplings (<2 m) with straight, unbranching stems preferred by males for performing courtship displays, as well as seven trees (2–4 m tall) for perching/resting. Fresh papaya and banana slices with liquid vitamin supplement were provided at four feeders twice a day, in the morning and at midday. A water bath was provided, cleaned and refilled daily.

We conducted observations on three groups of T-treated individuals, which included two groups of females ($N = 4$ females, 9–25 October 2014; $N = 3$ females, 19 November – 9 December 2015) and one group of juvenile males ($N = 4$ males, 25 November–14 December 2014). Observations of the first T-treated group of females were terminated after 17 days because one female aggressively monopolized access to food in the aviary; observations for all other groups were terminated after 20–21 days. During the

second experiment with T-treated females, one female was also more aggressive than the others, which restricted the other females' use of feeders, so we added 8–10 feeding locations throughout the aviary, which allowed the other females access to food in spite of increased agonistic interactions. We also observed two control groups (see treatment descriptions below, Treatment Administration), which included one group of females ($N = 4$ females, 1–21 September 2015) and one group of males ($N = 3$ males, 8–28 October 2015).

Sex Determination

Females and juvenile males are superficially indistinguishable from one another (olive green plumage), thus we determined each bird's sex using a molecular approach that involves amplifying homologous sections of the *CHD* gene, which is present on both Z and W chromosomes, but which differs in length. Products appear as one or two bands, respectively, for males and females (Griffiths et al., 1998). At capture, ~5–20 μl of blood was collected by puncture of the brachial vein and stored in 100 μl Queen's lysis buffer at 4 °C until analysis. DNA was extracted using the DNeasy Blood and Tissue kit (Sigma-Aldrich, St Louis, MO, U.S.A.) and the concentration was adjusted to 20 ng/μl. The *CHD* gene was PCR-amplified (at 62 °C annealing temperature), and PCR products were separated by electrophoresis. Samples were run in duplicate together with DNA from one or two males of known sex, or one known male and one known female.

Treatment Administration

Once birds were assigned to a treatment group, they were implanted at the base of the neck either with Silastic tubes (Dow Corning Inc., Midland, MI, U.S.A., 10 mm long, 0.76 mm inner diameter, 1.65 mm outer diameter) filled with crystalline T (Steraloids Inc., Newport, RI, U.S.A.), or with empty Silastic tubes as control. We followed the implantation procedure described previously (Fuxjager et al., 2012), where levels of T were elevated in T-implanted females on average 2.24 ng/ml as compared to levels in nonimplanted females of 0.51 ng/ml. In separate studies using different implants, male-like behaviours were detected in T-implanted birds 7 days post-treatment and peaked at day 13 (Day et al., 2007), so here, implanted birds were relocated to the outdoor aviary 7 days following implantation where they were observed for 21 days (17 days for one group, see below).

Courtship and Court-clearing Observations

Birds were introduced to the aviary between 0830 and 1200 hours and observed for 1–2 h to ensure that they fed. Afterwards, 4 h of behavioural observations were performed daily after the birds' morning food provision at ~0630 hours. An observer, sitting inside the double-door entrance to the aviary, recorded all instances of behaviour during each observation period. In addition, a tripod-mounted Sony Handycam CX220, set near the entrance, recorded each observation period, capturing activity in ~70% of the aviary. We documented the occurrence of previously described courtship behaviours such as roll-snaps, jump-snap displays, grunt-jump displays, which were the focus of another study (Chiver & Schlinger, 2017). For this study, we focused on observations of arena cleaning, which involves removing leaves or other dead plant materials from the court or biting and pulling at shoots or leaves of live plants. To ensure that plant material of different types was available for removal, and because leaves can become covered in mud during the rainy season, we augmented dead leaves throughout the aviary, twice during each experiment (~ every 7 days from the experiment's onset).

Weighted Leaves Removal Experiment

Reproductive drive can be assessed experimentally by an organism's willingness to endure discomfort or to engage in demanding tasks to gain access to a sexual partner in order to complete appetitive or consummatory sexual behaviours (Pfaff, 1999). To assess male manakins' motivation to clear their courts, we challenged wild males by placing weighted artificial leaves within their courts. We then recorded whether the males removed the leaves, the males' latency to remove a leaf, the weights of leaves removed, the number of attempts to remove the leaves and how much time males devoted to leaf removal. Males holding courts on 10 separate leks were presented with natural-looking (green) thin plastic leaves (115 mm long × 75 mm wide; 1.3 g) with or without added weight consisting of metal (wire and/or coins) taped to the underside of the leaf. For the first two males tested, we presented leaves in order of increasing weight (with a 2 g increase between presentations); afterwards, leaves were presented in random order. The complete set of artificial leaves included 15 leaves with weights: 1.3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, 36, 50 g. For comparison, adult male golden-collared manakins weigh on average 18 g (Brumfield, Jernigan, McDonald, & Braun, 2001). The experiment took place during the breeding season and shortly after (22 February – 14 July during both 2015 and 2016) when males were observed to attend their courts daily.

Leaves were presented every 2–3 days, between 0600 and 0700 hours, when males typically start displaying at their courts (Barske et al., 2014). The leaf was placed in the clear part of the arena, near the base of one of the saplings and a camera was set on a tripod ~1.5–3 m from the court to record display on the entire court. After 3–4 h, we checked to determine whether the leaf was removed. If the leaf was still present on the court the camera was left to record for an additional 2-h (total recording time 5.5 h). At retrieval, we noted whether the leaf was outside the male's display court, at the edge of the male's display court or inside the male's display court ('outside', 'edge' or 'inside', respectively) and, from the recording, we determined (1) when the male first approached and grabbed the leaf with their bill (approach time); (2) how the leaf was moved: (a) whether males flew with the leaf without touching the ground, (b) whether the leaf was dragged (including flipped), or (c) whether it was lifted and dropped; we also noted all other male behaviours directed at the leaf (e.g. flutter and/or pecking at the leaf) and display behaviour at the court; (3) the number of attempts (when males approached and grabbed the leaf in their bill, even if they did not subsequently move the leaf); (4) the duration of the jump-snap display bouts that involved attempts to move the leaf; and (5) the number of 'cheer' vocalizations given during the display bouts when the male approached the leaf. 'Cheer' vocalizations are typically used by males when reacting to an intruder or other threats at the court and in this context may indicate the male's perception of the leaf as a threat (McDonald, Clay, Brumfield, & Braun, 2001).

Statistical Analysis

To determine the effect of T on the daily occurrence of cleaning behaviour, we used a mixed-effects logistic regression analysis. We included sex, treatment, day in the aviary and day∗treatment interaction as factors with bird ID as a random variable. We initially included all factors in the model and assessed the contribution of each variable by comparing models with and without each variable using a chi-square test.

We used logistic regression analyses to determine whether the weight of the leaf influenced the males' method of leaf removal (flying with it versus dragging or lifting and dropping it) and where they moved the leaf relative to their display court (outside, edge, inside). We used linear models to examine factors influencing the duration of removal and the approach time (both variables were normalized by log transformation). We used a generalized linear model with a Poisson error distribution to examine factors affecting the number of attempts at removal and the number of 'cheer' vocalizations given during the experiment. We classified attempts at leaf removal into four groups (1, 2, 3, ≥4 attempts), but we obtained the same results when a logistic model (1 attempt versus ≥2 attempts) was used. For all analyses, we started with a model including all possible explanatory variables and compared it with increasingly simplified models. We determined the contribution of each variable (leaf weight, presentation order, etc.) by comparing models with and without the variable using a chi-square test. To determine whether differences between males contributed significantly to the variation in the measures of interest, we first used male ID as a factor in all models and, if not a significant contributor, then we included it as a random factor (mixed-effects model). Similarly, to determine whether the behaviour measured varied over the course of the trials, we included leaf presentation order as a variable in all models and omitted it from the final model if it was not significant. All statistical analyses were conducted in R, version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

T Activation of Court-clearing Behaviour

The probability of court clearing differed significantly between the sexes and increased significantly with T treatment (sex: $\chi^2_1 = 7.1$, $P = 0.0077$; T treatment: $\chi^2_1 = 4.0$, $P = 0.045$; Fig. 1). Over the course of 236 h of observations of females with and without T treatment, none were seen to set up courts or clear live or dead plants from the ground. T did activate some male courtship behaviour as well as considerable aggressive behaviours (Chiver & Schlinger, 2017). Even without T treatment, the three juvenile males set up courts and one was observed to pluck at a small herb on the court. All four males treated with T established courts and three of the four males were observed (total of 17 observations) to pluck at small herbs on the court and/or clear leaves from the court.

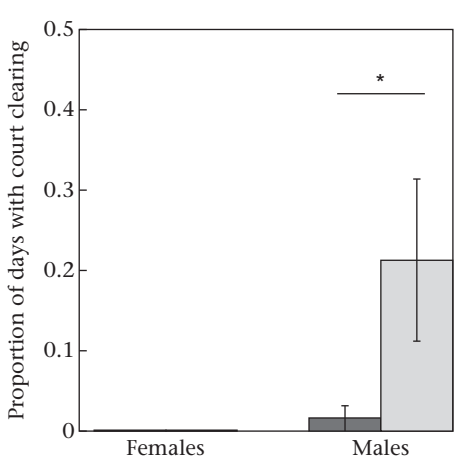

Figure 1. Proportion of days that individuals were observed to engage in court-clearing behaviour. Dark grey bars: blank-treated (control) individuals; light grey bars: testosterone-treated individuals.

Weighted Leaf Removal Experiment

Artificial leaves were presented at 27 courts held by adult males. Of these presentations, two males ignored the leaf and continued to perform courtship displays, 16 males abandoned the arena for the duration of the leaf presentation (~5.5 h) and 11 males (across 8 leks) removed the leaves. For 10 of the 11 males that removed the leaf, we continued to present leaves for a total of 60 leaves (the number of leaves in each of the 15 weight categories were as follows: 6, 9, 2, 6, 1, 5, 1, 8, 6, 3, 2, 5, 2, 2, 2). Each of these 10 males were presented with an average of 5.9 leaves (range 4–8). We also presented leaves to two males (presumably juveniles, with green plumage) who were observed displaying repeatedly at courts. These males abandoned their courts for the duration of the presentation.

For the males that removed the leaves, there was significant variation among males as to when they approached the leaf, with

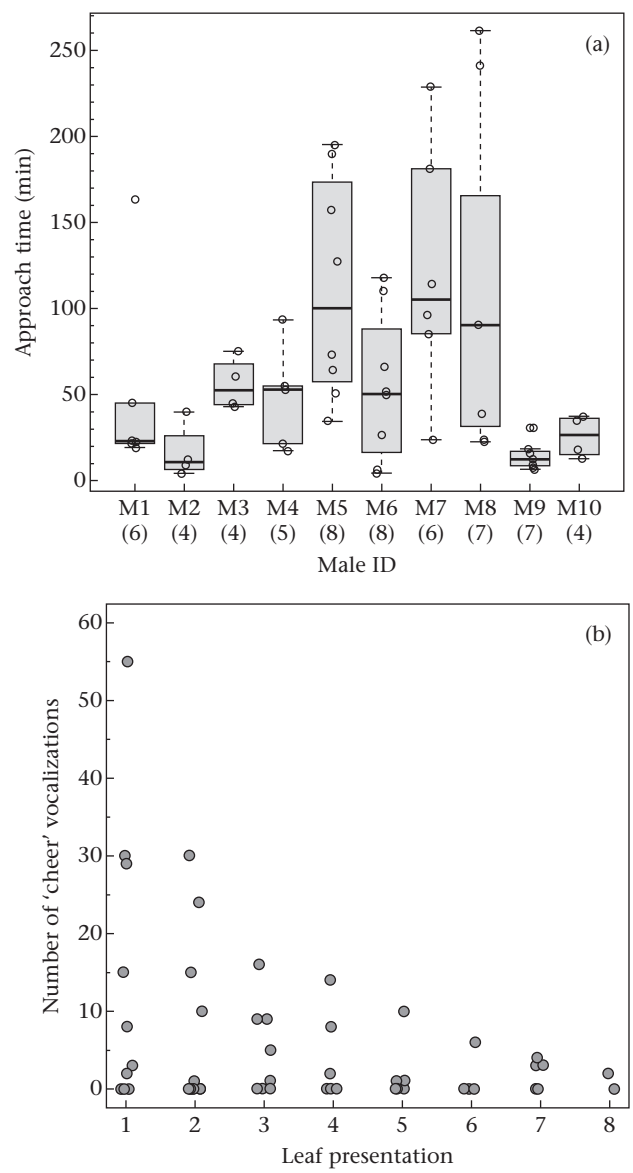

Figure 2. (a) Time to approach the artificial leaf from the start of the experiment for 10 of the 11 males (one male was presented only one leaf). Box plots include median, interquartile range and range. Individual observations are indicated by open circles and sample sizes are indicated in parentheses. (b) Number of 'cheer' vocalizations produced over the course of leaf presentation experiments.

about half of the males consistently approaching and removing the leaf within 1 h, and the remainder varying more widely in approach time (ANOVA: $F_{1,58} = 4.7$, $P = 0.03$; Fig. 2a). We found no significant differences between males in how they removed the leaf, the number of attempts, the duration of the leaf removal or the number of vocalizations uttered. The number of 'cheer' vocalizations decreased over the course of leaf presentations ($\chi^2_1 = 160.3$, $P < 0.001$; Fig. 2b) and the duration of the leaf removal also showed a slight decrease over the course of the experiment ($\chi^2_1 = 4.5$, $P = 0.03$).

The probability of leaf removal off the court decreased significantly with leaf weight ($\chi^2_1 = 58.01$, $P < 0.001$). All of the relatively light leaves, up to 8 g, were completely removed from the arena with the exception of one that was moved to the edge (Fig. 3a). Most of the moderately heavy leaves (10–16 g) were also completely removed from the arena (27%) or to the edge of the arena (67%), although one remained inside the arena. Leaves that exceeded the 18 g average body weight of a male manakin were also moved, but with more difficulty. Most leaves of 18–24 g were moved to the edge of the arena with only one (7%) left inside. The birds attempted to move even the heaviest leaves, although they were only moved to the edge or were left inside the arena. The heaviest leaf moved any distance was 36 g.

The males' method of leaf removal varied depending on the weight of the leaf ($\chi^2{}_1 = 47.15$, $P < 0.001$; Supplementary Video S1). For leaves weighing up to 8 g, the birds picked up the leaf and flew outside the arena before dropping it (Fig. 3b). Birds also picked up and flew with many leaves weighing 10–16 g, although a few were dragged or immediately dropped after being lifted. Some leaves weighing 18 g were also picked up and flown off the arena, however most were moved by being lifted and/or dragged. All leaves weighing more than 24 g were lifted and/or dragged. In addition, three males were observed on a total of four occasions to flutter above the leaf. The air movement beneath the wings can move unweighted leaves, so this strategy may be used to assess the weight of the leaf or to remove light debris. Four males on a total of seven occasions were observed to peck at the leaf vigorously, a strategy which may help to break up heavier objects and make them easier to carry off. However, males also pecked at leaves of intermediate weight (12–18 g) and pecking probability was not significantly associated with leaf weight ($\chi^2_1 = 0.052$, $P = 0.80$).

As the weight of the leaves increased, birds required more attempts and more time to remove them from their courts (attempts: $\chi^2_1 = 12.35$, $P < 0.001$; time: $\chi^2_1 = 14.16$, $P < 0.001$; Fig. 3c and d). For leaves weighing up to 8 g, birds usually completed the task in one attempt and in under 1 min. Leaves weighing 10–16 g could still be moved in one attempt and in under 1 min, but 47% required two or more attempts and 53% required 2 min or longer. A large percentage of the leaves weighing more than 18 g required more than one attempt and more than 1 min to remove: one male made 18

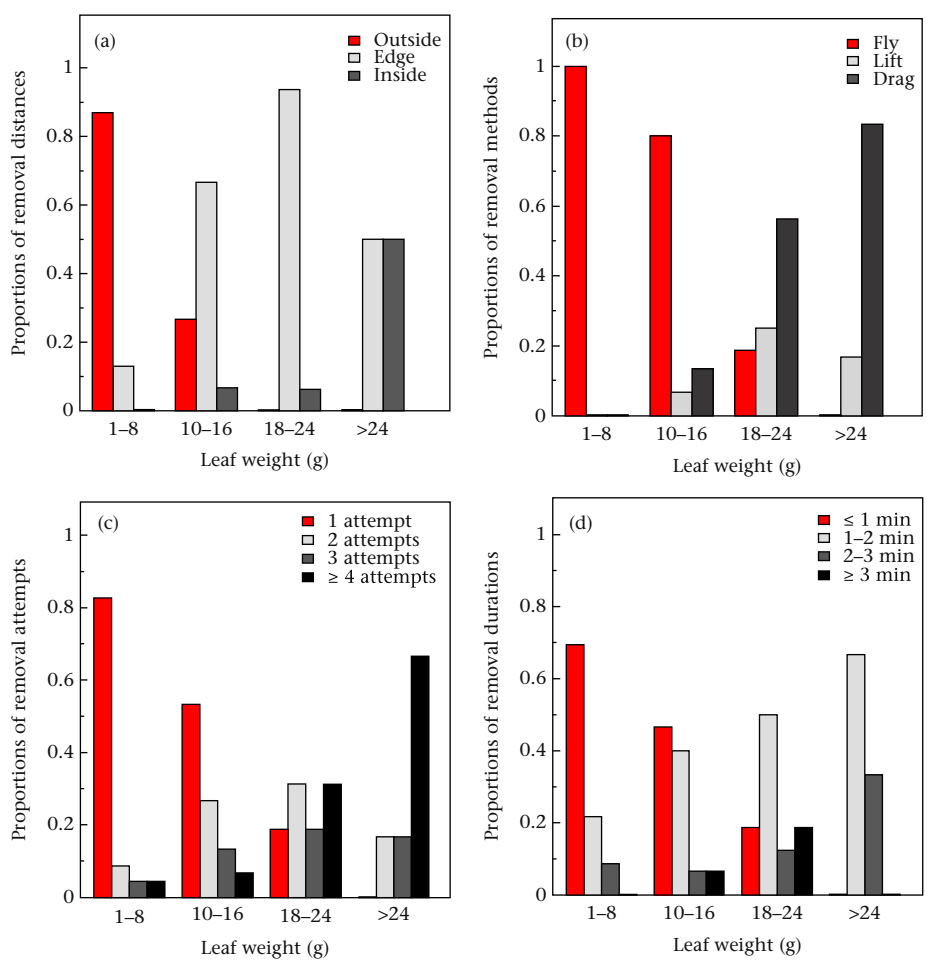

Figure 3. (a) Removal distances of artificial leaves relative to the male's display court (outside, edge, inside) as a proportion of observations for each weight class of leaf. (b) Proportion of leaf removal methods (fly, lift, drag) for each weight class of leaf. (c) Proportion of removal attempts (approach/grab) for each weight class of leaf. (d) Proportion of leaf removal durations (includes handling the leaf and displaying around the leaf) for each weight class of leaf.

attempts, taking 7 min. However, even for the heaviest leaves (18–24 g), 19% of the birds moved the leaf in one attempt and within 1 min.

DISCUSSION

Court cleaning by manakins involves a complex suite of behaviours that can be physically challenging. Females appear fully unmotivated to claim and maintain a court, and in the many hours of observations of female behaviour, we never observed females performing this behaviour, even after treatment with testosterone. Thus, the set of behaviours used to keep a court clean have undergone a rather rigid sexual differentiation that is likely based on specialized androgen-dependent neural circuits that are well developed in adult male golden-collared manakins but less developed, if at all, in adult females, much like a variety of other appetitive or consummatory reproductive behaviours in other vertebrates (McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012). Developmentally, testicular hormones can create sex differences in avian neural circuits (Adkins-Regan, 2013), although in birds and other vertebrates, nonhormonal actions such as those mediated directly by sex-chromosome-specific genes, can also influence sexually dimorphic behavioural phenotypes (Arnold, 2017; McCarthy et al., 2012). Future experiments whereby female manakins are treated with testicular hormones in ovo and then tested as adults for male behaviours, including court clearing, would be informative.

T treatment in juvenile males increased the motivation to engage in court clearing. In the wild, adult males experience elevated T levels just prior to the breeding season (Schlinger, Barske, Day, Fusani, & Fuxjager, 2013), indicating that the motivation for court clearing is activated naturally with age and reproductive maturity. The development of this behaviour in adults contrasts with some other behaviours, such as foraging or habitat choice, where the motivation to explore and interact with novel objects decreases with age likely as learning about new foods primarily takes place during the juvenile stages (Brunon, Bovet, Bourgeois, & Pouydebat, 2014; Greenberg, 2003). Court cleaning in manakins does, however, resemble behaviours of bowerbirds, in which juvenile males also respond to T treatment by increasing practice of bower construction (Collis & Borgia, 1992; Wingfield & Borgia, 1991). However, the complexity of the display and bower structure also increases with age, pointing to an important role for experience, as males learn from being assessed by visiting females and juveniles (Borgia & Keagy, 2015).

Juvenile males naturally exhibit some court-cleaning behaviour, although in our observations of two wild juvenile males at courts, clearing was limited to the base of one sapling and the courts appeared smaller than those of adult males. Because juvenile males have an olive green plumage, clearing the court to emphasize plumage coloration may not be an important selective force at this stage. Court clearing by juvenile golden-collared manakins may be important in claiming a territory and facilitating interactions with other juvenile and adult males at the lek, as seen in other manakins where young males establish dominance hierarchies and queue for a position at the lek (McDonald et al., 2001). This may imply that androgens in male golden-collared manakins could act to influence sensory or cognitive abilities. For example, adult males might become more attuned or attentive to the amount or quality of light reaching or reflecting from the display court, thus stimulating leaf removal. The court shape and size might be perceived as a feature of a desired structure or landscape that is disturbed by the leaf. For other behaviours, such as song, changes in context associated with different hormonal states could indicate that androgens recruit additional brain regions involved in assessment and/or performance of the behaviour (Alward, Balthazart, & Ball, 2013; Apfelbeck et al., 2013). Perhaps sex hormones affect visual processing or other neural circuits to influence cleaning behaviour.

In addition to being assessed based on their plumage and courtship display, females may also judge males based on the quality of their display court, with males maintaining a clear court favoured for matings. Novel behaviours, and their genetic underpinnings, are established based on benefits accrued through positive reinforcement (Tebbich, Griffin, Peschl, & Sterelny, 2016). As for other behaviours that involve responses to environmental cues, such as food or habitat selection (Greenberg, 2003), golden-collared manakin males may have a template, possibly activated by androgens, which then guides the general features to which males are attentive. In the wild, there is variation among adult males in court size and shape (Chapman, 1935), suggesting that the template, if innate, may be flexible and adjusted during the juvenile period, possibly as they observe and learn from adult males at their courts.

With the addition of weights, leaf removal became a more difficult task but one that many of the males were motivated to complete. Some males did not attempt to remove the leaf, but those that did often made numerous attempts, spent a significant amount of time and used different strategies for removal. Thus, not unlike other appetitive behaviours that serve to achieve access to a receptive mate (Balthazart, Reid, Absil, Foidart, & Ball, 1995; Pfaff, 1999), male manakins exhibit a considerable drive to keep their courts clear. There were, however, considerable differences in court-clearing performance of the males chosen for study. These differences could reflect individual variation in fearfulness of exploring novel problems, reproductive drive or a bird's age and/or experience removing debris (Cummings, Clinton, Perry, Akil, & Becker, 2013).

The variety of object shapes and sizes that males have to remove from their courts over the course of the breeding season likely presents a challenge. Males in our study typically made more attempts and spent more time attempting to remove heavier leaves from their courts. Males were also more likely to remove heavy leaves ($\geq$28 g) by dragging them or lifting and dropping them rather than by flying with them. Many birds are exceptionally well adapted for flight by, among other traits, concentrating the bulk of their body weight beneath and between their wings (Gill, 2007). Some flying birds are also adapted for carrying weights above their typical body mass. Prior to performing extended migrations, some migrating birds can add substantial fat reserves up to 50% of the premigratory body mass (Odum & Connell, 1956). Raptors of all kinds can carry relatively large prey items relative to their own body size. In these cases, the extra weight is positioned to retain the bird's centre of gravity beneath and between the wings (e.g. prey items are carried in talons and held beneath the wings; fat depots and muscle mass of the flight muscles are concentrated in the bird's abdomen and thorax; e.g. Heers & Dial, 2012). Male golden-collared manakins pick up and carry objects of equal or greater weight than their own body weight and they do so by picking up and carrying the objects in their beaks. Thus, the male must fly with considerable mass positioned well away from its centre of gravity, very likely a difficult task. Leaves can also be quite large and awkward, increasing the difficulty of the task (Chapman, 1935). Some birds do fly while carrying relatively large items in their beaks, including prey items (e.g. terns and alcids) or materials used for nesting. These items weigh substantially less than the bird and, while still difficult to transport in flight, they do not require the amount of lifting accomplished by court-cleaning manakins. Thus, in addition to muscle adaptations that seem to underlie the motorically complex and powerful wing- and roll-snaps (Fuxjager, Goller, Dirkse, Sanin, & Garcia, 2016; Schultz, Hertel, Bausch, & Schlinger, 2001),

manakins may also require androgen- and perhaps sex-specific muscular capabilities for lifting the material that might obscure their display arenas. The successful employment of this new motor pattern may have facilitated the elaboration of court-cleaning behaviour in *Manacus* manakins. Male golden-collared manakins are indeed stout birds (Lowe, 1942), but more work is needed to assess anatomical and physiological adaptations that enable manakins to lift and carry such large masses in their beaks.

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Supplementary Material

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