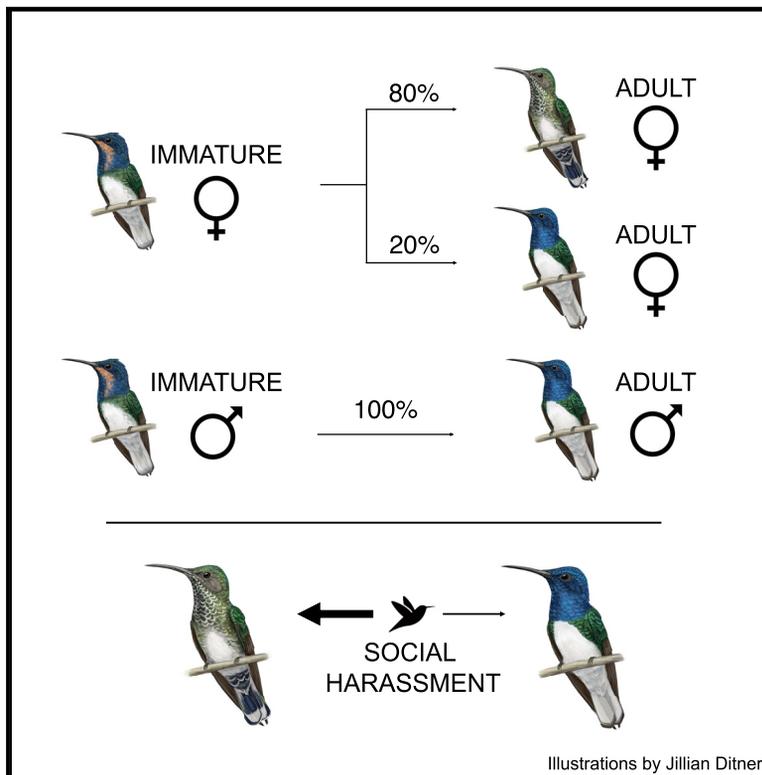


Current Biology

Male-like ornamentation in female hummingbirds results from social harassment rather than sexual selection

Graphical abstract



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In brief

Female white-necked jacobin hummingbirds are polymorphic—some express ornamented plumage like adult males. Falk et al. find that all juvenile females have male-like plumage, which some maintain as adults. Rather than being favored by sexual selection, male-like plumage benefits females by reducing social harassment from this and other species.

Highlights

- Female white-necked jacobins are polymorphic—30% have male-like plumage
- All juveniles have male-like plumage, excluding sexual selection as an explanation
- Male-like females receive less con- and heterospecific social harassment
- Thus, male-like ornamentation in females can arise purely through non-sexual means

Report

Male-like ornamentation in female hummingbirds results from social harassment rather than sexual selection

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SUMMARY

Ornamentation is typically observed in sexually mature adults, is often dimorphic in expression, and is most apparent during breeding, supporting a role for sexual selection in its evolution.^{1–4} Yet, increasing evidence suggests that nonsexual social selection may also have a role in the evolution of ornamentation, especially in females.^{5–9} Distinguishing between these alternatives remains challenging because sexual and nonsexual factors may both play important and overlapping roles in trait evolution.^{7,10} Here, we show that female ornamentation in a dichromatic hummingbird, the white-necked jacobin (*Florisuga mellivora*), cannot be explained by sexual selection. Although all males are ornamented, nearly 30% of females have male-like plumage. Remarkably, all juveniles of both sexes express ornamented plumage similar to adult males (androchromatism), but 80% of females acquire non-ornamented plumage (heterochromatism) as they age. This unique ontogeny excludes competition for mates as an explanation for female ornamentation because non-reproductive juveniles are more likely to be ornamented than adults. Instead, avoidance of social harassment appears to underlie this female-limited polymorphism, as heterochrome taxidermy mounts received more aggressive and sexual attention than androchrome mounts from this and other hummingbird species. Monitoring electronically tagged birds at data-logging feeders showed that androchrome females accessed feeders more than heterochrome females, presumably because of reduced harassment. Our findings demonstrate that ornamentation can arise purely through nonsexual social selection, and this hypothesis must be considered in the evolution of not only female-limited polymorphism but also the spectacular ornamentation often assumed to result from sexual selection.

RESULTS AND DISCUSSION

Although ornamentation is often explained by sexual selection,^{2,4,11} social competition for resources—whether used for breeding or for reasons unrelated to reproduction—also can favor the evolution of elaborate traits, especially in females.^{5,7,12} Direct tests of the social selection hypothesis can be challenging because intraspecific variation in female ornamentation is rarely discrete, and interpretation of interspecific comparisons can be limited.^{13,14} Female-limited polymorphism in which some females resemble males (androchromes) and other do not (heterochromes) occurs in a variety of taxa, including damselflies,^{15,16} lizards,¹⁷ butterflies,¹⁸ fish,^{19,20} birds,²¹ and other lineages.^{22,23} In birds, studies of museum specimens suggest that female-limited polymorphism may be particularly common in hummingbirds, where a large proportion of sexually dimorphic species appear to contain polymorphic females, both as juveniles²⁴

and as adults.^{25,26} Because the complete lack of male parental care in hummingbirds suggests that sexual selection in females is likely to be weak, alternative explanations such as nonsexual social selection may play a role in the evolution of female ornamentation.²⁴ Although socioecological factors have been hypothesized to explain female-limited plumage polymorphisms in hummingbirds,²⁵ the adaptive function of male-like plumage in females remains unknown because all previous studies have been limited to examinations of museum collections.^{25–27}

To determine whether sexual or nonsexual social selection underlies female-limited polymorphism and male-like ornamentation, we first described the frequency and ontogeny of color variation in wild-caught individuals of both sexes in a widespread hummingbird species, the white-necked jacobin (*Florisuga mellivora*). We then observed hummingbird responses to taxidermy mounts to test predictions of both hypotheses. Finally, we quantified the potential fitness effects of female ornamentation



Figure 1. Plumage types of the white-necked jacobin (*Florisuga mellivora*)

White-necked jacobins are depicted in a perched position (top) and a fanned open tail (bottom). Heterochromes (left) are all female and distinguished by completely green dorsums, mottled gray and green throats and chests, and dark green and black retrices fringed with white on the outer retrices. Androchromes (center, right) have shiny blue heads and throats, green backs, white chests, bellies, neck spots, and retrices fringed with a black outline. Juveniles of both sexes are androchromic (right) with a buff facial stripe and wide tail band typically only seen when the tail is fanned. Adult androchromes (center) may be male or female, but adult female androchromes typically have a wide tail band similar to juveniles rather than the fine edge illustrated here, which is typical of males. Illustrations by Jillian Ditner. See also [Figures S1](#) and [S2](#) and [Table S2](#).

by monitoring the foraging behavior of free-living white-necked jacobins using passive radio-frequency identification (RFID) tags and a network of tag-detecting feeders. For ornamentation to adaptively benefit androchromic females through sexual selection, females must express ornamented male-like plumage during reproductive life stages, and males must prefer ornamented females as mates. Alternatively, if nonsexual social selection in the form of social harassment—either detrimental sexual attention or social aggression—explains the presence of this trait, we expect to see a reduction in harassment toward androchrome females that results in increased access to resources.

To test these alternative hypotheses, we captured 436 white-necked jacobins (including 135 recaptured in at least one subsequent year) in Gamboa, Panama ($9^{\circ}7'12''$ – $79^{\circ}42'0''$) between July 2015 and June 2019. Based on their plumage, 324 individuals were scored as androchromic, 107 as heterochromic, and 7 as mixed plumage type ([Figure 1](#)). This scoring method matched unsupervised clustering of extracted color values from photos of 208 birds in all but three individuals that were classified as mixed plumage type during capture ([Figure S2](#); [STAR Methods](#)). Genetic sexing of 401 individuals scored for plumage revealed that all males and 28.6% of females were androchromic. Thus, all male white-necked jacobins appear to be androchromic, but females can be either color type ([Figures 1](#) and [S1](#)).

After characterizing the prevalence of female-limited polymorphism in white-necked jacobins, we explored the ontogeny of female plumage coloration. Bill corrugations can be used to age hummingbirds, as more corrugation is found on immature than adult birds.^{28–30} We found that the percentage of bill corrugation was predictive of female plumage type (accuracy = 0.83, 95% confidence interval [CI] = [0.73, 0.88], $p = 0.0003$). Most notably, females with the highest amount of corrugation (60%–80%, $n = 14$) were all androchromic, whereas only 20.2% of females with little corrugation (0%–40%, $n = 104$) were androchromic ([Figure 2](#); [STAR Methods](#)). These results indicate an ontogenetic shift for most females from ornamented androchromatism when young to non-ornamented heterochromatism when older, as has been suggested previously for another hummingbird

species.²⁴ Yet, ~20% of females retain androchromic plumage as adults, a result confirmed by photographic and video evidence of androchromic females incubating and tending nests (see [STAR Methods](#), Androchrome females as reproductive adults). This unusual ontogeny of female color change was further supported by the recapture of two females that were androchromic as juveniles, but heterochromic the following year when captured as adults. In contrast, when we captured three androchromic females as adults, all retained their androchromic plumage when recaptured in subsequent years. Females captured in heterochromic plumage were always recaptured in the same plumage type in subsequent years ($n = 17$). Thus, plumage type shifts can occur during the immature period, but there is no evidence that shifts occur in adulthood.

To explore the potential function of female color differences, we observed hummingbirds' initial interaction with pairs of taxidermy mounts on feeders that differed in sex and/or plumage type. We presented three combinations of taxonomic mounts: (1) heterochrome female versus androchrome male (Hf-Am: different sex/different plumage), (2) heterochrome female versus androchrome female (Hf-Af: same sex/different plumage), or (3) androchrome female versus androchrome male (Af-Am: different sex/same plumage) ([Figure 3](#)). Because hummingbirds compete intensely with both con- and heterospecifics for nectar resources,^{31,32} we recorded sexual and aggressive behaviors ([Table S1](#)) of all hummingbird species that interacted with mounts. Sexual selection could only explain androchrome plumage in adult females if they are preferred as mates, which would be indicated by sexual behaviors directed toward androchrome female mounts. Alternatively, social harassment—either as detrimental sexual attention or aggression toward heterochrome mounts—could favor the evolution of ornamentation in females via nonsexual social selection.⁹

In contrast to predictions of the sexual selection hypothesis, our mount experiment revealed that males exhibit a clear sexual preference for heterochrome rather than androchrome females. If heterochrome female mounts were present in a trial (Hf-Am and Hf-Af), the first incidence of sexual behavior was directed toward heterochrome female mounts in 100% of trials ([Figure 3](#)).

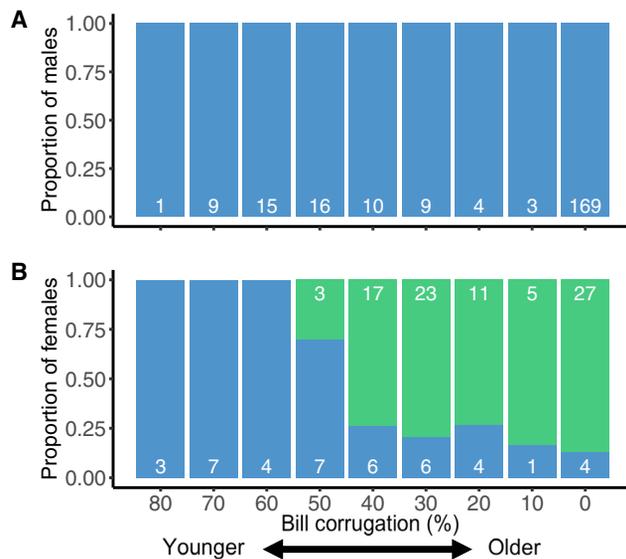


Figure 2. Plumage type in relation to age

Proportion of bill corrugation can be used to gauge age, with high corrugation corresponding to younger age in both (A) males and (B) females. Each bar shows the proportion of individuals with a given corrugation level appearing in androchromic (blue bars) versus heterochromic coloration (green bars). Number of androchromes is listed on the bottom of each bar, and heterochromes on the top. All males, regardless of age, are androchromic. Young females are entirely androchromic, whereas older females are mostly heterochromic. This pattern indicates an ontogeny involving a shift from androchrome to heterochrome in most but not all females. Subtle variation in androchrome tail and facial pattern were also predicted by corrugations and can be used to distinguish age and sex in some cases (see [Figure 1](#); [STAR Methods](#)).

However, we found no directional preference when both mounts were androchromic (Af-Am) ([Figure 3](#)).

Aggression from interacting hummingbirds was also more often displayed toward heterochrome females in trials in which they were present (Hf-Am and Hf-Af trials) ([Figure 3](#)), rather than toward androchrome females or males. In trials with both female and male androchrome mounts (Af-Am), aggression was unbiased toward mount type ([Figure 3](#)). To rule out the possibility that some sexual behaviors from males could have been misinterpreted as aggression, we removed data from androchromes that interacted with mounts and found qualitatively similar results (Hf-Am, $n = 14$, $p = 0.057$; Hf-Af, $n = 18$, $p = 0.001$; Af-Am, $n = 6$, $p = 1.00$). In addition, we found that hummingbirds avoided aggression toward androchrome female mounts in Hf-Af trials even when white-necked jacobins that interacted with mounts were removed from the dataset, leaving only heterospecifics ($n = 15$, $p = 0.007$). Although sample sizes were too small ($n < 10$) to test only heterospecific interaction with mounts in Hf-Am and Af-Am trials, the proportion of individuals behaving aggressively toward each mount type did not differ between white-necked jacobins and other hummingbird species in any of the trial types (Hf-Am, $p = 1.00$; Hf-Af, $p = 0.45$; Af-Am, $p = 1.00$). Overall, mount color type played a greater role in predicting aggression than did mount sex, with heterochrome female mounts receiving more aggression than either androchrome

female or male mounts, a result consistent with the nonsexual social selection hypothesis.

These patterns of aggressive behavior toward mounts are consistent with observations of chases in the wild. During 78 h of video-recorded observation of mount trials, we noted every in-frame displacement chase, including those involving an androchrome, a heterochrome, or another species. In the 269 chases involving at least one androchrome, we found that androchromes were more often chasing rather than being chased (1.45 chaser:chasee ratio). In contrast, in the 90 chases involving at least one heterochrome, heterochromes were chased more than 10 times as often as they chased others (0.08 chaser:chasee ratio). Therefore, androchromes were far more socially aggressive than heterochromes ($\chi^2 = 81.04$, $p < 0.0001$). Although the sexes of aggressive androchromes were unknown in these observations, both white-necked jacobins and other hummingbird species might avoid social aggression by limiting attacks on androchromes, which is consistent with our findings from the mount experiment that male-like plumage allows females to avoid social harassment.

To determine whether avoidance of social harassment by androchrome females might result in a fitness benefit, we monitored the feeding behaviors of birds (36 heterochrome females, 15 androchrome females, and 103 males) with implanted RFID tags and recorded their presence at 28 tag-detecting feeders dispersed across an ~ 0.84 km² area in Gamboa. To mimic natural variation in floral nectar quality, we maintained half of the feeders with high sugar concentration (1:3 ratio sucrose:water), and half with low concentration (1:6 ratio sucrose:water). Mixed-effects models were used to analyze feeding data ([Table S3](#); [STAR Methods](#)).

In 88,528 feeding visits over 278 days, birds visited feeders more often when they were maintained with high sugar concentration ($p = 0.02$), but bird plumage type was not a significant predictor of visiting these high-sugar feeders ([Figure 4A](#); [Table S5](#)). In contrast, the estimated feeder visit frequency of androchrome females was higher (potentially driven by a higher daily feeder visit probability) ([Figure 4B](#); [Table S5](#)) and the estimated mean feed durations were longer than those of heterochrome females ($p = 0.009$ averaging across high- and low-sugar feeders) ([Figure 4C](#); [Table S5](#)). Moreover, the feed duration of heterochrome females interacted with sugar concentration ($p < 0.0001$), such that their feed duration was shorter at high-sugar feeders, making the difference between female plumage types larger at high-sugar feeders ([Figure 4C](#); [Table S5](#)). Taken together, androchrome females accessed feeders both more frequently and for longer durations than did heterochrome females, a pattern most pronounced at preferred food resources where competition was likely highest.

Sexual selection has long offered a rich theoretical background for understanding biological ornamentation and sexual dimorphism.^{2,33,34} Yet, we found that the majority of female white-necked jacobins exhibit male-like ornamentation only during their non-reproductive life stage, when sexual competition does not occur. Similarly, an examination of another hummingbird species found that more juveniles than adult females had variable numbers of androchromic gorget feathers.²⁴ In addition, male white-necked jacobins showed a stronger sexual preference for heterochromes than for androchromes in mount observations. Thus, sexual selection cannot explain

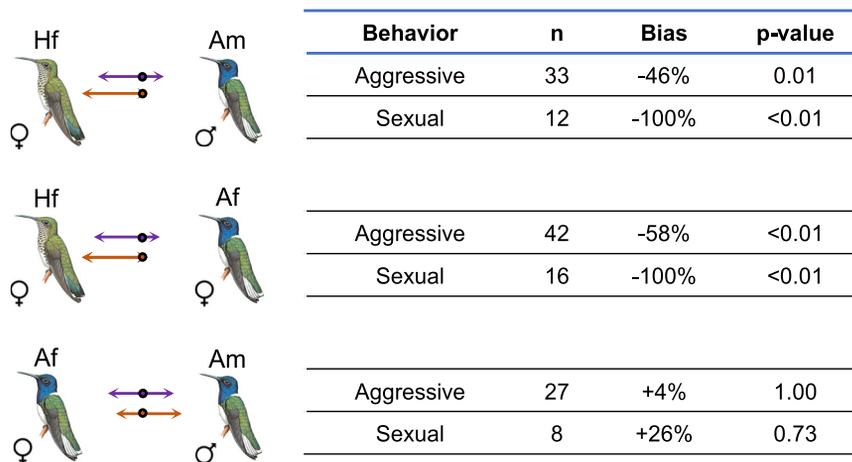


Figure 3. Behavior toward taxidermy mounts

Taxidermy mounts (Hf, heterochrome female; Am, androchrome male; Af, androchrome female) were presented in the wild on hummingbird feeders in three pair types (left panel). We recorded first interactions of aggressive and sexual behaviors for each distinguishable hummingbird (by species, sex, or color type). Bias indicates the percent of interactions toward the mount type depicted on the left minus the percent bias for the mount type depicted on the right. Therefore, negative values indicate behaviors were more often directed toward the mount type on the left, and positive values indicate behaviors more often directed toward the right. Bias magnitude indicates preference skew in either direction. p values were calculated with binomial tests and indicate the probability that there is no bias in either direction. Arrows between mounts also show bias in aggressive (purple), and sexual (orange) behaviors. The skew of the arrow to either side of the dot represents the percent of bias toward a mount type. Illustrations copyright of Lynx Edicions.

the evolution of male-like female ornamentation in this dichromic species.

What then explains the presence of ornamented male-like plumage in females of this and other hummingbird species? Ornamentation in young birds has been shown to evolve due to parent-offspring interaction, but such traits are typically present at hatching, differ from adult coloration in either sex, and are significantly or completely replaced at independence.^{35–40} Because androchromic ornamentation in white-necked jacobins does not follow these ontogenetic patterns, this explanation cannot explain the presence of androchromic features in non-reproductive juveniles.^{41,42} White-necked jacobins maintain androchromic plumage after fledging (and in some individuals, through adulthood), suggesting that the trait may be adaptive to females because it allows them to escape social harassment. Social harassment could take the form of excessive or detrimental sexual attention from males, as has been suggested in some other species exhibiting female-limited polymorphism,^{15,43,21} or by exclusion from food resources. Consistent with the idea that male-like plumage enables females to escape sexual attention, male sexual behavior was first directed toward heterochrome mounts in every case in which one was present. However, during 26 3-h trials of the mount experiment, we recorded only 136 instances of sexual behavior toward the mounts (28 toward heterochrome females, 28 toward androchrome females, and 80 toward androchrome males) and none between wild birds. Moreover, courtship was rarely witnessed at any time of year, including the breeding season, even though white-necked jacobins were extremely common. Although sexual attention does not appear to be frequent or otherwise harmful to white-necked jacobin females, we remain cautious in rejecting this hypothesis because sexual attention may fluctuate seasonally, and because distinguishing sexual from aggressive behavior is challenging. Detrimental sexual attention and aggressive interactions are both disruptive from a female's

perspective and can be viewed as non-mutually exclusive components of social harassment.

Although sexual interactions were infrequent, aggressive interactions with both white-necked jacobins and other hummingbird species were common. During the 26 trials of the mount experiment, we witnessed 1,790 instances of aggression toward mounts (510 incidences toward heterochrome females, 529 toward androchrome females, and 751 toward androchrome males), and 359 chases involving wild white-necked jacobins. Aggression was therefore frequent, and androchrome plumage effectively reduced these encounters. Female plumage color also appeared to affect foraging behavior, and therefore likely fitness, as RFID data of feeding behaviors showed that androchrome females had increased access to food resources, likely the result of reduced social harassment at feeders. Social competition for access to food resources may therefore be the ultimate driver of ornamentation in female white-necked jacobins and other hummingbird species. This hypothesis is supported by the finding that the feed duration advantage of androchrome females over heterochrome females was greater at preferred food resources where competition with other hummingbirds would have been higher. Although the data presented here cannot determine whether androchrome plumage is an honest signal of high resource holding potential in both sexes, androchrome plumage itself appears sufficient to deter aggressive interactions.

The source of selection is a notable distinction between these hypotheses. Whereas sexual attention will primarily come from conspecifics, social aggression may come from any individual that overlaps in niche space with white-necked jacobins, regardless of species. This idea is consistent with our observation that both white-necked jacobins and other hummingbird species appeared to avoid aggression toward androchrome females and is important given the high frequency of interspecific interactions in hummingbirds.^{44–47} The relative frequency of sexual versus aggressive harassment—as well as con- versus heterospecific

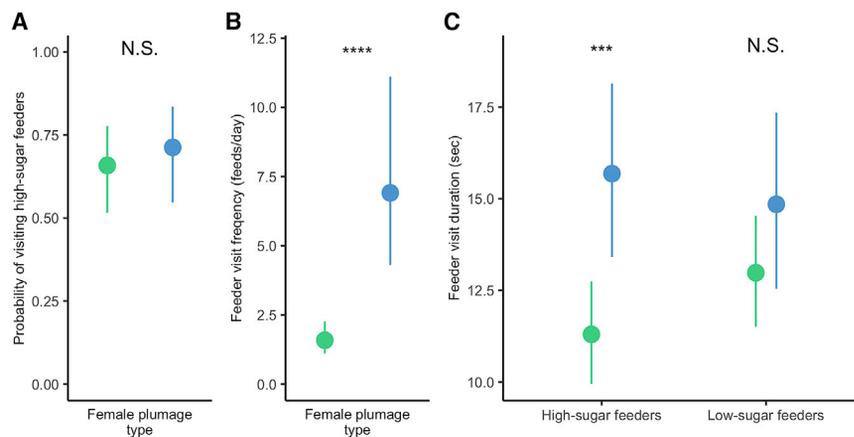


Figure 4. Feeding behaviors of heterochrome and androchrome females

Estimated means and 95% confidence intervals are indicated for heterochrome females (green circles and lines) and androchrome females (blue circles and lines). p values for differences in estimated means are indicated by N.S.: $p > 0.05$, ***: $0.001 > p > 0.0001$, **** $p < 0.0001$. All values were estimated using mixed-effects models.

(A) Models of the probability of visiting high-sugar feeders were not improved by incorporating plumage type as a factor.

(B) When including days both with and without feeds, androchrome females had a higher feeder visit frequency than heterochromes.

(C) Androchrome females accessed feeders for longer durations, but this was most apparent at high-sugar feeders due to an interaction effect.

See also [Tables S3](#), [S4](#), and [S5](#).

harassment—may shift across seasons and across the lifetime of an individual.

Finally, although we focus primarily on females in this study, the ornamented plumage found in male juveniles is also unusual. The social harassment hypothesis applies equally to juvenile males and females and therefore may explain why young birds of both sexes are ornamented in this and other hummingbird species. Ultimately, social harassment from sympatric hummingbird species offers an intriguing potential explanation for why female-limited polymorphism has evolved repeatedly and frequently in the hummingbird clade.²⁵ Last, it is unclear why most adult females are heterochromic, given that we found little advantage to this plumage type. Heterochrome females were strongly preferred as mates, but there is no evidence that female hummingbird fitness is limited by access to matings. Alternatively, these non-ornamented females may experience reduced predation while nesting, as has been suggested more broadly for the evolution of unornamented plumage in female birds,⁴⁸ or the benefit of androchromatism may be frequency-dependent.

Females of many species are as brilliantly ornamented as males,^{48,49} and variation in female ornamentation exceeds that seen in males in many hummingbird species, including this one. The plumage ontogeny of white-necked jacobins demonstrates that, even in highly dimorphic animals that are likely to be under strong sexual selection, intrasexual competition for mates does not provide a complete understanding for the evolution of ornamentation, particularly in females. Instead, social harassment from both males and females, as well as from con- and heterospecifics, over access to critical food resources appears to explain the existence of female ornamentation in the white-necked jacobin, and possibly other hummingbird species.²⁶ In short, androchrome females avoid harassment while feeding, enabling them to access food more than those with non-ornamented plumage. For species with the highest mass-specific metabolic demands of any vertebrate,⁵⁰ selection on traits that maximize feeding in the face of intense resource competition from any other bird—regardless of sex or species—may explain why female-limited polymorphism has evolved so often in hummingbirds,²⁵ yet so rarely in other avian species. Thus, considering the roles of sexual and nonsexual

competition, both within and even among species, is critical for understanding the evolution of exaggerated traits in both sexes.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2021.07.043>.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.J.F., M.S.W., and D.R.R.; Methodology, J.J.F.; Formal Analysis, J.J.F.; Investigation, J.J.F.; Writing – Original Draft, J.J.F.; Writing – Review & Editing, J.J.F., M.S.W., and D.R.R.; Visualization, J.J.F.; Funding Acquisition, J.J.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
White-necked jacobin presence at RFID feeders	This paper	Zenodo https://doi.org/10.5281/zenodo.5035295
Color values extracted from photos	This paper	Zenodo https://doi.org/10.5281/zenodo.5035295
Capture database and genetic sexing data	This paper	Zenodo https://doi.org/10.5281/zenodo.5035295
Mount experiment data	This paper	Zenodo https://doi.org/10.5281/zenodo.5035295
Software and algorithms		
ImageJ	51	https://imagej.nih.gov/ij/
Image Calibration and Analysis Toolbox 1.0	52	http://www.jolyon.co.uk/myresearch/image-analysis/image-analysis-tools/
R software	53	https://www.r-project.org
R package MASS	54	https://cran.r-project.org/web/packages/MASS/MASS.pdf
R package <i>emmeans</i>	55	https://cran.r-project.org/web/packages/emmeans/index.html
R package <i>lme4</i>	56	https://cran.r-project.org/web/packages/lme4/index.html
R package <i>cluster</i>	57	https://cran.r-project.org/web/packages/cluster/index.html
R package <i>caret</i>	58	https://cran.r-project.org/web/packages/caret/index.html
R package <i>lmtest</i>	59	https://cran.r-project.org/web/packages/lmtest/index.html
R package <i>glmmTMB</i>	60	https://cran.r-project.org/src/contrib/Archive/glmmTMB/
Other		
RFID Tags	Biomark	BIO8.B.03V1
RFID Detector and Data Logger	Dr. Eli Bridge, University of Oklahoma	https://onlinelibrary.wiley.com/doi/10.1111/j.1557-9263.2010.00307.x
Hummingbird Feeder	Aspects	Item #153
Hummingbird Feeder	First Nature	993051-001
Camera	Samsung	EV-NX1000BABGB

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Jay Falk (j.jinsing@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Data and code are available at Zenodo: <https://doi.org/10.5281/zenodo.5035295>. This includes capture information, genetic sexing data, color values extracted from photos, mount experiment data, RFID data, and the R code used to analyze each dataset.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

White-necked jacobins (*Florisuga mellivora*) are a wide-ranging hummingbird species primarily found in Central and South American lowland tropical regions. Two subspecies are recognized. The more populous subspecies (*F. mellivora mellivora*) can be found distributed from its northern-most limit in southern Mexico to its southern-most limit in Peru, northern Bolivia, and northern Brazil. All data in this study derive from this subspecies, collected in the town of Gamboa, Panama and surrounding region (9°7'12," –79°42'0"). The second subspecies (*F. mellivora flabellifera*) is limited to Tobago island in the Republic of Trinidad and Tobago.

METHOD DETAILS

Sampling

White-necked jacobins were captured by placing mist nets next to hummingbird feeders, or in mesh drop-traps placed around feeders. Blood samples (5–15 ul) were collected with a 75 ul glass capillary tube by puncturing the tarsal vein with a 27-gauge needle and stored at room temperature in vials containing 0.25 mL of 2% SDS lysis buffer.

Genetic Sexing

At the Cornell Lab of Ornithology, all DNA samples were extracted using QIAGEN DNeasy Blood & Tissue Kits. We used 2550F/2718R primers to amplify fragments of the sex chromosomes,⁶¹ but were unable to achieve adequate amplification and visualization on 2% agarose gels for some individuals. For those individuals that we could not sex clearly with this approach, we used 1237L/1272H primers⁶² with fluorescently labeled 1237L. Amplified fragments were detected with an Applied Biosystems 3730xl sequencer and analyzed using the Peak Scanner module on Thermo Fisher Connect. We verified the accuracy of both methods using frozen tissue from three male and three female white-necked jacobins from the L.S.U. Museum of Natural Sciences Collection that had been sexed by gonad morphology (specimens B-37138, B-37139, B-71913, B-71964, B-2641, B-4927).

Plumage Color Photos

All birds were photographed in a 25x25x25 cm diffuse light box lined with white felt fabric. LED strips (12V battery-powered weatherproof IP66 6500K 76 lm/ft) were fixed to the top and bottom edges to reduce iridescent effects through use of omnidirectional and diffuse lighting. We used a Samsung NX1000 mirrorless digital camera with a 20–50 mm lens (maximum zoom, ISO 100, f/20) fixed to the top of the box to photograph all birds. Birds were photographed in hand at the bottom of the box with a gray background in frame with six gray standards from an X-Rite ColorChecker Passport. We photographed each bird at six angles: dorsal head, throat, right-lateral head, neck, ventral closed tail, and ventral spread tail. Three photographs of each angle were taken with bracketed shutter speeds, and the brightest of these with no oversaturation was chosen for analysis.

Mount Observations

We observed how birds interacted with taxidermized mounts to understand whether and how color differences in white-necked jacobins might mediate behavioral interactions. Two heterochrome females, four androchrome females, and four males were collected and sexed by gonad morphology during dissection at the Smithsonian Tropical Research Institute Bird Collection, and then later confirmed through genetic sexing. All specimens were prepared in a perched position with folded wings by Jorge L. Medina. We avoided the use of androchrome male and female specimens with buff facial stripes since this plumage is likely associated with immaturity (see [Quantification and statistical analyses: Identifying Features of Immaturity](#)).

The three types of mounts were presented in pairs, resulting in three types of trials: (1) heterochrome female versus androchrome male (Hf-Am); (2) heterochrome female versus androchrome female (Hf-Af); or (3) androchrome female versus androchrome male (Af-Am). From February through May 2017 and 2018, we ran 180 min trials between 07:00–11:30, and from February through May 2019 we ran two 75 min trials per day between 07:00–11:30. To avoid repeated sampling of the same birds, we spaced each trial in a different location at least 50 m apart and with no line of site from one location to the next. This restriction limited the number of available spaces in which it was possible to do trials, so we repeated locations no less than two months apart. Tropical hummingbird feeding territories are thought to shift continuously depending on nectar availability and plant flowering times,⁶³ so it is unlikely that the same birds inhabited the same areas across this time span.

During each trial, we hung Aspect HummZinger Mini hummingbird feeders from two 2.75 m poles spaced 3 m apart. Each feeder was filled with 200 mL of sugar-water (1:4 sugar to water ratio by volume). We randomly chose mounts from each type, then flipped a coin to determine the feeder on which each mount would be placed. We fixed mounts to the center of the feeder in a perched position

facing away from the pole so that approaching birds would see the mount when approaching. Videos of trials were recorded with a wide angle at 60 frames per second. To ensure accurate identification of “bird type” (i.e., heterochrome female, androchrome female, or androchrome male), we also watched the feeders and recorded behaviors with binoculars from a > 10 m distance.

We recorded the species and sex, when identifiable by plumage (see above), of each bird that interacted with a mount. For white-necked jacobins, we recorded color type rather than sex. Individual birds could not be identified, so we recorded the feeder and its associated mount for aggressive and sexual behavior of each distinguishable bird for each trial (Table S1). Since most birds could not be distinguished beyond species, sex, or color type, behaviors for each of these “distinguishable types” was recorded only once per trial. On rare occasions, androchrome white-necked jacobins could be distinguished by a wide tail stripe so these were included as separate data points.

Finally, for 26 trials from 2017, we used BORIS v4.1.1⁶⁴ to log all behavior events (Table S1) for the entire 180 min trial. This included interactions with the mounts, but also included every chase interaction between individuals within frame. For chases, we recorded the species, sex, or color type of both the chasing bird and the bird being chased. We tallied the number of chases in which each bird type (i) chased another hummingbird and (ii) was chased by another hummingbird. Chases where a bird was chased by the same type were counted as an instance of both chasing and being chased.

Observing Feeding Behavior with RFID

To assess the feeding behavior of individual white-necked jacobins, we used an RFID system to log feeding behavior at feeders set up across Gamboa. White-necked jacobins were subdermally tagged with Biomark HPT8 PIT tags between the shoulders. To detect tagged birds in the wild, we modified First Nature 16 oz. Hummingbird Feeders to attach the RFID loggers⁶⁵ and a 51.5 mm circular antennae over a single nectar access hole. All other holes were blocked. Loggers were set to read three times per second, with each read lasting 0.2 s. If a tag was detected the ID was logged with date and time to the second. To prevent reads from logging while a bird perched without feeding, we prevented perching by removing the landing ring around the feeder. Tags could be detected 3–4 cm from the center of the antenna loop in the direction of an incoming hummingbird, and roughly 0.5 cm from the outside of the loop. We verified the accuracy of this system before deploying feeders in the wild by testing three birds in a flight cage (two heterochrome females, one male). Immediately after tagging, we released the birds into the cage for 90 min. The logger detected the tag in every feeding event and did not log during any other behavior.

We began RFID tagging white-necked jacobins in December of 2017, tagging most subsequent captures until the experiment ended in May 2019. All birds were genetically sexed as described above. Feeders were placed a minimum of 60 m apart with no line of site between any two feeders, and we attempted to keep spacing even. Half of the feeders were maintained with high, and half with low sugar concentration (1:3 versus 1:6 ratios sucrose:water by volume). RFID data were collected from January through May in 2018 and again from October 2018 through May 2019 (Table S4), but only data from January through May were used in both years to restrict data to the dry season. Each feeder’s sugar concentration was switched once per year. Feeders were randomly assigned high and low sugar concentration in equal numbers in January of 2018 and the assignments were switched on April 2. In January 2019 we again randomly assigned sugar concentration and switched assignments on April 8. Each feeder was examined for functionality and adequate sugar water on a daily basis except Sundays and were refilled with fresh sugar water at least 3 times per week, or daily if sugar water was low during a check. Each feeder was washed with soap and water once per week. Loggers were powered with 12V, 7 Ah lead-acid batteries which were recharged every two weeks.

Permissions

All experimental procedures were approved by Institutional Animal Care and Use Committees (IACUC) at both Cornell University (2009-0105) and the Smithsonian Tropical Research Institute (2015-0618-2018-A1, 2016-0120-2019, 2017-0116-2020). Collections were permitted by the Panamanian Ministry of the Environment under SE/A-84-14, SE/A-11-16, SE/A-15-17, SE/A-100-17, and SE/APHBO-7-18. Blood samples were exported from Panama to the U.S.A under C.I.T.E.S. permits SEX/A-101-15, SEX/A-111-16, SEX/A-73-17, SEX/A-47-2018, SEX/A-36-19, and U.S.D.A. Import Permit 52686. All collected specimen skins are a part of the Smithsonian Tropical Research Institute Bird Collection in Panama City, Panama.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data summaries, graphing, and shaping were done using the tidyverse package in R.^{66,53}

Plumage Color Analysis

All birds were classified in-hand by J.J.F. into three categories: androchrome, heterochrome, or mixed. We compared this subjective categorization with objective unsupervised categorization of color values from photographs of 208 birds. To extract color information from photographs we used ImageJ⁵¹ and Image Calibration and Analysis Toolbox 1.0, which measures reflectance, color, and pattern objectively and to animal vision.⁵² Photographs were standardized and linearized using three gray standards in each photo (3.1, 36.2, and 90.1% reflectance). We chose three regions of interest to analyze: dorsal head, ventral head, and ventral closed tail. Although photographs of other regions were taken, we chose these three regions to fully analyze to reduce redundancy between regions, and because these were the least prone to measurement error (e.g., shadows cast by the hand hold, or difficulty in defining the boundaries of region of interest). Regions of interest were outlined by individuals who were blind to the sex of the birds.

The top of the head included the entire dorsum of the head and ended posteriorly at the distinctively larger feathers of the shoulder region. The throat region included the entire ventral side of the head and ended posteriorly at a lateral line between both wrist joint (wings were held folded naturally against the body in all photographs). Ventral closed tail included all retrices folded naturally, and undertail coverts. For each region of interest, we extracted mean red, green, and blue pixel values, resulting in nine color variables for each individual. One outlier was removed from the analysis because piloerection resulted in considerable reduction in reflectance. We then performed a principal component analysis with all variables using the *MASS* package in R.^{53,54} The first and second principal components accounted for 82.1 and 11.1% of the variation, respectively. All other dimensions accounted for < 4% of variance and eigenvalues < 0.5 (Table S2).

To determine the structure of color variation in this species, we used cluster analyses to determine whether individuals cluster in coloration, and if so, the degree of clustering. We first used the k-means algorithm⁶⁷ to calculate the total within-cluster sum of squares for 1-10 potential clusters using all nine axes from the PC analysis. An elbow plot illustrates a large drop after $k = 1$ and bend at $k = 2$ clusters (Figure S2), indicating two clusters within the data. We also calculated average silhouette width⁶⁸ for the same potential clusters using the *cluster* package in R^{53,57} and found the highest average width when $k = 2$ (Figure S2). After concluding that the data clustered into two groups, we assigned each individual bird to a cluster using the k-means algorithm.⁶⁷ All birds identified as androchromes fell into one cluster and all identified as heterochromes fell into the second cluster. For those labeled as “mixed,” two clustered with androchromes and one with heterochromes, making objective descriptions of these individuals difficult without photographic analysis. The results of this analysis demonstrate that color is indeed polymorphic in this species. The 100% accuracy of field identification of color type for androchromes and heterochromes gave us confidence that those identified without photographic analysis were likely accurate. Unsurprisingly, mixed plumage types were not reliably classified into either cluster, but this identification was very rare, with only 7 of 558 (1.3%) captures labeled as such.

Ultraviolet Reflectance

Although some hummingbirds can detect wavelengths < 400 nm, which are not visible to humans,^{69,70} the degree of hummingbird UV detection capability is debated and may vary among species.⁷¹ Nevertheless, we analyzed UV reflectance from specimens of white-necked jacobins from collections at The American Museum of Natural History to determine the degree of UV reflectance, and whether measuring UV reflectance would be valuable in classifying plumage morphs in this species. We used five males, five heterochrome females, and five androchrome females that were sexed by gonad. All were photographed in visual (red, green, and blue) and UV (uv-R, and uv-B) channels with a Canon EOS 7D, 50mm lens at $f/0$, ISO 400 in a diffuse lighting arena. We included Labsphere Spectralon gray standards at 2, 20, 80, and 99% reflectance in all photographs.

No additional plumage patterns were revealed in UV channels. We compared UV reflectance from the top of the head, throat, and ventral closed tail using Image Calibration and Analysis Toolbox (see Plumage Color Analysis for details). The tail appeared to display the most UV reflectance. However, overall correlation of UV and visual channels was high, in particular with the blue channel (0.98 correlation between uv-R and blue, 0.98 between uv-B and blue). Similar results were found with the throat region (0.98 between uv-R and blue, 0.97 between uv-B and blue). UV reflectance is therefore largely redundant with blue reflectance in these regions. The top of the head showed a different pattern such that visible and UV reflectance had lower correlation (0.53 between uv-R and blue, 0.46 between uv-B and blue). However, the reflectance of UV in this region was low and similar between males (4.53%, SEM = 0.10%), androchrome females (4.59%, SEM = 0.07%), and heterochrome females (4.46%, SEM = 0.15%). Thus, UV reflectance is unlikely to be a distinguishing factor between sexes or color types in this species. We therefore opted to not take UV photographs with birds in the field given the difficulty of collecting both UV and visual reflectance from live animals with limitations on handling time.

Distinguishing Sex with Color

Androchromes may be male or female, but subtle differences within this plumage type may be able to predict sex. We trained a discriminant function analysis in R^{53,54} using color values from photographs of androchromes and their known genetic sex. We then used a permutation analysis to determine whether color values can assess genetic sex better than random. After generating a confusion matrix for the observed data using predicted values from the discriminant function, we permuted the sex of androchrome males and androchrome females and generated confusion matrices 5000 times. We then compared the accuracy of the observed model versus permuted models. In both female ($p < 0.0002$) and male ($p < 0.0002$) androchromes, color predicted sex better than permuted models. However, sex was predicted with jack-knifed models at 95.5 and 63.6% accuracy for male and female androchromes respectively, showing that while better than random, accuracy in distinguishing sex this way is low.

Bill Corrugation Estimation and Accuracy

Hummingbird bills are highly corrugated when the birds are young, making it a useful aging tool.^{28–30} J.J.F. estimated the degree of corrugation to the nearest 10% on all captured birds from the distal end of the nasal groove to the tip of the bill. In 2019, after estimating corrugation proportion, we additionally measured the corrugation with calipers (± 0.05 mm) to assess the accuracy of estimations. Of 133 measurements, 71 had > 0% corrugation. We assessed the accuracy of estimations in two ways. First, we measured the distance between true and estimated percentages. True corrugation percentage was different from the estimate by a mean difference of 3.53 percentage points (SD = 2.95) when excluding individuals with no corrugation, and 1.88 percentage points (SD = 2.78) if these individuals were included. Measurements and estimates were correlated in individuals with corrugations ($R^2 = 0.94$,

$p < 0.0001$) and across all individuals ($R^2 = 0.98$, $p < 0.0001$). Second, we reduced the precision of the measured percentage by rounding to the nearest multiple of 10% and compare with the estimations. Estimates matched the rounded measured percentage in 80.3% of cases with corrugation, and 89.5% overall. In those that did not match, estimates never exceeded a 10% difference from the rounded measurement. Thus, estimates and measurements of corrugation are largely similar.

Identifying Features of Immaturity

Androchromes occasionally had a buff facial stripe and/or a wide stripe on the distal part of the rectrices that resembled the darker heterochrome tail in coloration (Figure 1). During capture of androchromes, we marked whether a face stripe was present or absent; face stripes were never present on heterochromes. For each sex, we used a logistic regression to determine whether corrugation level predicted facial stripe presence. If a significant relationship existed, we measured accuracy of the model using the *caret* package in R.⁵⁸

Our data suggest that these traits are associated with immaturity, with the exception of the wide tail stripe in females. In both females and males, corrugation level is predictive of the presence of a buff facial stripe (females: $p = 0.001$, accuracy = 0.82, 95% CI = [0.68, 0.92]; males: $p < 0.0001$, accuracy = 0.93, 95% CI = [0.85, 0.95]), suggesting that androchrome plumage with a facial stripe likely indicates immaturity in both sexes. Among androchrome females, 12/14 (86%) of individuals with 60%–80% bill corrugation had buff facial stripes, while only 5/21 (24%) of individuals with 0%–40% bill corrugation expressed this plumage. Corrugation level is also predictive of a wide tail stripe, but only in males (males: $p < 0.0001$, accuracy = 0.94, 95% CI = [0.88, 0.97]; females: $p = 0.68$). In females, a wide tail stripe was found on nearly all androchromes (42 of 45). Thus, in most cases, adult androchrome females may be identified by the presence of a wide tail stripe and the absence of a buff facial stripe.

At its high and low extremes, bill corrugation percentage can be used to distinguish first year immatures from adults.^{28,29} However, the threshold degree of corrugation at which individuals can be accurately aged often varies by species.²⁸ Data from mark-recapture studies can help identify these thresholds if birds can be recaptured across multiple years. In our study, we compiled data from all 135 white-necked jacobins that had been recaptured in at least two different years. No bird was ever recaptured with > 50% bill corrugation; only one retained 50%, and four retained 40%. Therefore, in this species, individuals with > 50% bill corrugation are almost certainly in their first year.

Androchrome females as reproductive adults

Capture data suggests that all female and male juveniles have androchrome plumage like adult males. Bill corrugations and recaptures indicate that most females then molt to heterochrome plumage as adults. However, our data suggests that ~20% of females retain androchrome coloration into adulthood. The retention of androchrome plumage in some reproductive adult females is supported by the photos and video evidence of nesting androchromes, linked below. In hummingbirds, nesting behavior is exclusive to reproductive females. A lack of buff facial stripe also indicates that the subjects in all these media are likely adults (STAR Methods: Identifying features of immaturity).

These media of nesting androchrome female white-necked jacobins are archived in the Cornell Lab of Ornithology Macaulay Library.

Photos:

<https://macaulaylibrary.org/asset/52899121>

<https://macaulaylibrary.org/asset/108412741>

<https://macaulaylibrary.org/asset/107789431>

Video:

<https://macaulaylibrary.org/asset/488778>

Mount Observation Analyses

Once aggressive and sexual behaviors were compiled for each of the three trial types, we calculated the proportion of times each distinguishable type directed their first sexual and aggressive behaviors toward different mount types. Only the first sexual and aggressive behaviors for each distinguishable type were used because repeated mount interactions may or may not have involved the same individual, so we would not be able to tell whether we were recording repeated or independent choices. In addition, repeated interactions with the non-responsive mounts would likely result in habituation, which we could not account for. Androchrome white-necked jacobins, heterochrome white-necked jacobins, and rufous-tailed hummingbirds (*Amazilia tzacatl*) interacted with mounts the most. Since other species were rare when treated separately, we grouped them together. We then used a Fisher's exact test to find if different distinguishable groups directed their first aggressive behavior to mounts in different proportions. This step was unnecessary for sexual behavior because only androchrome white-necked jacobins exhibited these behaviors. If we found no evidence of group independence, we compiled all incidences of aggression or sexual behaviors and used a binomial test to see if the behavior of interacting birds deviated from the null expectation of equal interaction rates at the two feeders regardless of mount type. Lastly, we also used a Fisher's exact test to test whether white-necked jacobins directed their aggressive behavior to mounts in different proportions than other hummingbird species.

Analysis of RFID data

Males and heterochrome females have never been shown to shift their plumage types, but androchrome females may shift to heterochrome plumage. Two females that were androchromes in 2018 were also detected in 2019, but since we were unable to recapture them to identify whether they have shifted color type, their 2019 visits were removed from the analysis. Another two androchrome females from 2018 were recaptured in 2019 and we verified that they had molted to heterochrome plumage. Therefore, we changed their plumage type to heterochrome in the 2019 RFID dataset. Two females identified as mixed plumage type in 2018 were removed from the dataset because these birds cannot be consistently categorized (see Plumage Color Analysis) and only account for a very small portion of females (3.7%).

When hummingbirds visit a food source, nectar consumption is not continuous. Rather, hummingbirds often consume for several seconds, hover in the vicinity of the food source, then return to feeding and will cycle between these behaviors multiple times before leaving or perching. To distinguish between separate visits to the feeder versus visits with small gaps between consumption, we set a threshold by which gaps of longer periods than the threshold would be considered separate visits. To determine this threshold, we first compiled all gaps ($n = 278,099$) in the dataset between reads at the same feeder within the same day. As expected, most gaps were very short, but ranged up to the length of the day (45821 s). One-second gaps accounted for 41.5% of gaps, and the cumulative explanatory power of adding additional gap durations decreases with each additional second. Above 7 s, the increasing explanatory power of additional seconds was never above 1%. Therefore, we chose 7 s as the limit to gaps within the same visit. The duration of a visit is therefore the sum of time consuming nectar and small gaps of 7 s or less. To ensure results were not spurious for this threshold, we repeated statistical analyses with 5–12 s gaps. However, we report the complete results only from analyses using a 7 s threshold. We recognize that this cutoff is somewhat arbitrary, but simply separating visits with all gaps inflates the number of visits to a feeder beyond biological relevancy.

To test whether birds preferred high-sugar feeders, we calculated the number of times each feeder was visited per day. We then compared the mean visits per day during each of the 28 feeder's high-sugar versus low-sugar periods using a Wilcoxon signed rank exact test for paired data. Next, we tested whether feeding behaviors differed between the three types of birds (i.e., heterochrome female, androchrome female, and androchrome male). We analyzed five metrics of feeding behavior: feeder visit frequency (feeds/day) including days without feeds, feeder visit frequency (feeds/day) excluding days without feeds, daily feeder visit probability, feeder visit duration (seconds), and the probability of visiting high-sugar feeders (Tables S3 and S5). For feeder visit frequency including days without feeds and daily feeder visit probability, data for each individual were bound by first and last days of their presence in the dataset. During different periods of the experiment, the number of feeders available ranged from 6 to 28 from January 2018 through May 2019 (Table S4), which was included in the model as a fixed effect in all analyses. We used *glmmTMB* (negative binomial models) and *lme4* (logistic and linear models) packages in R to fit mixed-effects models^{56,60} (Table S3). For the feeder duration analysis, a square-root transformation of visit duration best fit assumptions of normality and homoscedasticity but estimated means and confidence intervals are presented with a back-transformation for easier interpretability. To verify that bird type was a relevant variable in determining each metric, we fit all models with and without bird type, and compared these two models with a likelihood ratio test using the *lmtest* package⁵⁹ in R (Table S5). If the two models were significantly different, we estimated marginal means, 95% confidence intervals, and pairwise contrasted p values using the *emmeans* package⁵⁵ in R with a Tukey adjustment for repeated within-family p value measurements (Table S5). Bird type was an important variable in all metrics except for the high-sugar feed probability, so we did not test pairwise contrasts for this metric. For simplicity, we only presented results for heterochrome and androchrome females in the main text, but all results including males can be found in Table S5.