Beyond plumage: acrobatic courtship displays show intermediate patterns in manakin hybrids

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ARTICLE INFO

A major goal of evolutionary biology is to understand how sexual traits arise and diversify among populations. One way to address this objective is by studying sexual traits in closely related species and their hybrids. Here, we used this approach to study the evolution of elaborate behavioural display characteristics used during courtship. We focus on bearded manakins (genus: *Manacus*), where males of this avian clade perform an acrobatic jump-snap display to court females. Hybridization is common among *Manacus* taxa, and thus, we studied courtship dance behaviour in a hybrid population between golden-collared manakins, *Manacus vitellinus*, and white-collared manakins, *Manacus candei*. We found that, despite being genetically more similar to white-collared manakins, hybrid males performed key dance manoeuvres like golden-collared manakins. Hybrids performed other dance manoeuvres intermediate to the two species, or more like their white-collared parents. Select components of the birds' dance routines may have therefore introgressed from the golden-collared population into the white-collared populations. We hypothesize that such modular evolution occurs in response to sexual selection, whereby specific components of the bird's dance routine shift to yield a broader change in its functional appearance.

Interplay between hybridization and sexual selection has played a major role in advancing our understanding of how reproductive traits arise and potentially shape speciation (Baldassarre et al., 2014; Baldassarre & Webster, 2013; Lipshutz et al., 2019; Parchman et al., 2013). The reason is that traits used to mediate behavioural processes like courtship are thought to help generate premating barriers that promote reproductive isolation and thus restrict gene flow between diverging populations, likely in concert with other factors (Cooney et al., 2017; Hudson & Price, 2014; Maan & Seehausen, 2011; Panhuis et al., 2001; Ritchie, 2007; West-Eberhard, 1983). Evidence in support of this view is mixed, such that some studies report correlations between assortative mating and species differences in sexual traits, whereas other studies do not (Brelsford & Irwin, 2009; Cooney et al., 2017; Hudson & Price, 2014; Kirschel et al., 2009; Lipshutz et al., 2017; Seddon, 2005; Sætre et al., 2003). However, much of this work explores sexual traits through a narrow lens by considering only one dimension of trait variability, i.e. plumage colour, acoustic signals, etc. (e.g. Brelsford & Irwin, 2009; Hudson & Price, 2014; Zonana et al., 2019). In reality, traits that are used to mediate reproductive interactions, such as courtship, are incredibly complex, incorporating multiple signals that function through a wide range of sensory channels and that interact with the environment in unexpected ways (Higham & Hebets, 2013; Mitoyen et al., 2019). Indeed, there is a long list of biotic and abiotic factors that can affect these signals and how they are perceived through space and time. It is therefore unsurprising that biologists so often find that variation in one component of a display is insufficient to explain assortative mating in nature. Addressing this gap is challenging, and one way to approach the problem is by expanding our study of sexual signals.

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We take this approach by comparing an elaborate dance display among populations of two closely related bearded manakins and the hybrids they produce in a small island population in western Panama. Bearded manakins include four distinct species within the genus *Manacus* (Family: Pipridae), which geographically replace each other across the lowland rainforests of South and Central America. The ranges of two species in particular, golden-collared manakins, *Manacus vitellinus*, and white-collared manakins, *Manacus candei*, overlap in the northwestern corner of Panama, where they interbreed to produce hybrids. This population (see map in Parsons et al., 1993) has been studied extensively for several decades, with ample research describing its genetic and phenotypic structure (Bennett et al., 2021; Brumfield et al., 2001; McDonald et al., 2001; Parchman et al., 2013; Parsons et al., 1993; Stein & Uy, 2006a; Uy & Stein, 2007; Yuri et al., 2009). Genomic indices of the hybrid population are skewed towards their white-collared manakin parents, yet sexually dimorphic plumage traits have likely introgressed into the hybrid population from the golden-collared population (Brumfield et al., 2001; Parchman et al., 2013). These traits include collar and belly colour such that hybrids maintain a pale yellow collar and (sometimes) a yellow belly; hence, hybrids are often called lemon-collared manakins (Parsons et al., 1993). This means that certain golden-collared manakin traits have merged into the hybrid population, creating discordance between the genetic and plumage (morphological) clines. Elevated male aggressiveness is another trait that seems to have introgressed asymmetrically from the golden-collared manakin population across the hybrid population (McDonald et al., 2001). Because both yellow plumage and male aggression are positively associated with reproductive success (Shorey, 2002; Stein & Uy, 2006a), these effects of introgression are often attributed to strong sexual selection by both female choice and male–male competition (Bennett et al., 2021; McDonald et al., 2001; Prado et al., 2009; Stein & Uy, 2006b). The distribution of the hybrids even stretches to many of the small islands that are immediately off the eastern cost of Panama near this contact zone (Bennett et al., 2021; Whetmore, 1972). Although the genetic architecture of these island birds is less clear, they are likely not entirely isolated from mainland birds (Bennett et al., 2021). Indeed, the islands themselves are within ≈500 m to 1.5 km of the mainland, and *Manacus* are known for their ability to fly over large bodies of water compared to other passerines that are similarly sized (Moore et al., 2008). Moreover, the island birds strongly resemble hybrids both in terms of plumage and other display traits (e.g. chee-poo call; Billo, 2011).

Research on hybrid populations of golden-collared and white-collared manakins has not yet addressed the birds' most spectacular and iconic display behaviour: their elaborate courtship dance (Chapman, 1935; Lill, 1974). All *Manacus* species have lek mating systems, in which males aggregate during the breeding season (January–July) to build courtship arenas (≈1 m²) and perform an elaborate display called the jump-snap display (Fig. 1; see Supplementary Video S1). Accordingly, males rapidly leap (not fly) among saplings that surround their arena, and in mid-air during these leaps, males snap their wings together above their backs. To produce these jumps, the male bird's strong leg muscles provide an acceleration of about nine times his mass to produce a series of perfect ballistic trajectories (Janisch et al., 2021). The result is a complex dance sequence, in which a small bird (≈20 g) bounces among saplings over a clear patch of forest floor, while generating loud firecracker-like pops that penetrate the forest habitat. In between each jump within a single jump-snap sequence, the male will land on a sapling feet-first. He then rapidly assumes a statuesque posture, in which he stands still with his elongated neck feathers erected to produce an expanded 'beard' in the direction of the female. Females normally evaluate the male dance from above; if they want to copulate with the male, they then join the display and perform a duo-dance (Barske et al., 2011, 2015). The elements of this coordinated behaviour are the same, but in this case, a female leads the displays by flying among the saplings, while the male follows and produces jump-snaps (Barske et al., 2015). It is thought that females provide males with a motor challenge, potentially to better assess their condition or quality (Barske et al., 2015; Fusani et al., 2014). Importantly, the fine details of the displays have been revealed by studies performed in the last 15 years using novel technologies that allowed filming the behaviour with high-speed cameras and later analysing it in slow motion (Barske et al., 2011; Fusani et al., 2007; Janisch et al., 2021). These studies
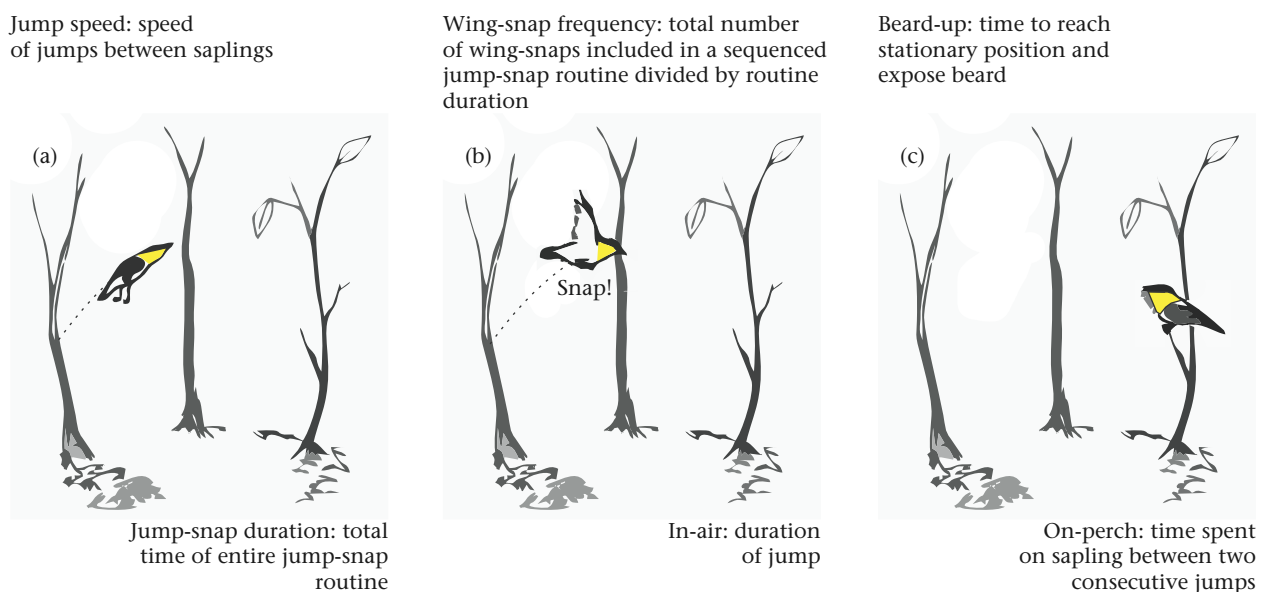

Jump speed: speed of jumps between saplings

Wing-snap frequency: total number of wing-snaps included in a sequenced jump-snap routine divided by routine duration

Beard-up: time to reach stationary position and expose beard

(a)

(b) Snap!

(c)

Jump-snap duration: total time of entire jump-snap routine

In-air: duration of jump

On-perch: time spent on sapling between two consecutive jumps

Figure 1. Schematic of the jump-snap display in *Manacus* manakins. (a–c) A single jump-snap manoeuvre depicted as a sequence. An entire jump-snap routine typically repeats this behavioural sequence several times in a row. High-speed behavioural measures recorded in the present study are denoted in each panel. Definitions of these behaviours are modelled after Barske et al. (2011).

revealed behavioural complexity and individual differences in movement patterns associated with mating success (Barske et al., 2011). A previous study on the effects of female presence on display performance, which included both white-collared and golden-collared manakins, revealed differences in the fine aspects of the display between the two species (Barske et al., 2015), although the study of interspecific differences was not the aim of the work. Interestingly, some authors have expressed the hypothesis that the plumage is a neutral trait irrelevant to male mating success and that the key trait for intersexual selection is the behavioural display (Butlin & Neems, 1994). To date, the display of the hybrids has not been studied quantitatively.

Here, we assessed whether measures of this dance behaviour differ among populations of golden-collared, white-collared and hybrid manakins from an island population. As the genetic background of this latter group of birds was rarely studied, we first confirmed that it is similar to mainland hybrids (and thus also similar to white-collared manakins). Next, we compared elements of the bird's dance display among the aforementioned groups. Because sexual selection has supposedly driven the asymmetrical introgression of plumage and behavioural traits from the golden-collared manakin population into the white-collared population, we predicted that island hybrids would likely produce courtship displays that resembled golden-collared manakins. Indeed, golden-collared manakins and white-collared manakins perform many elements of their dance displays and other courtship behaviours at different speeds, even if the quality of the overall dance is relatively similar (at least to human observers) (Barske et al., 2015; Miles, Goller et al., 2018). Moreover, these same core display features that differ among taxa play a role in mate choice, with field studies in golden-collared manakins showing that females preferentially mate with males that perform a variety of display manoeuvres at millisecond-faster speeds (Barske et al., 2011). This includes variables such as the time it takes males to assume the statuesque posture on saplings between successive jumps (beard-up time), the length of time males remain in this posture (on-perch time) and/or the frequency of wing-snaps during an entire jump-snap sequence (see Supplementary Video S1). Female preference for specific display traits has not been investigated in white-collared manakins. If hybrids perform displays similar to those of golden-collared manakins, and these are less preferred by female white-collared manakins compared to those of their conspecific males, then the behavioural components of courtship could counteract the preference for yellow/golden collar trait of golden-collared manakins and represent a force contrasting the expansion of the latter trait.

METHODS

All appropriate governmental and institutional authorities approved this research, including the Autoridad Nacional del Ambiente of the Republic of Panama, the Smithsonian Tropical Research Institute (STRI), the Organization of Tropical Studies (OTS) at La Selva Biological Station in Costa Rica and the Institutional Animal Care and Use Committee of the University of California, Los Angeles (IACUC protocol number: ARC 2009-123). We made every effort to minimize stress for any bird that was captured to collect blood and DNA samples, while all behavioural recordings were collected in a noninvasive manner via passive observation (see below).

Genetic Data

We explored genetic similarity between hybrid birds from the Panamanian mainland and Isla Bastimentos, where we collected our behavioural data (see below). Accordingly, we assessed patterns of genetic differentiation at nine microsatellite loci among multilocus genotypes across different manakin populations, specifically looking at allele size variation for loci AC2, AC8, AC10, AC12, AC13 and TCA1 from Brumfield (1999), Man3 and Man7 from Piertney et al. (2002) and LTMR8 from McDonald and Potts (1994), as described in Fusani et al. (2018). Our analyses focused on four populations: (1) white-collared manakins from the south bank of Rio Sixaola ≈25 km west of Guabito ($N = 2$; 9°31′48″N, 82°41′24″W); (2) golden-collared manakins from the Valiente peninsula ($N = 20$; 9°7′12″N, 81°54′W); (3) hybrid birds near Tierra Oscura on the mainland of Panama ($N = 12$; 9°11′24″N, 82°11′24″W); (4) hybrid birds from the Island of Bastimentos itself ($N = 9$; 9°18′0″N, 82°7′48″W). Based on previous studies exploring the population genetic structure of the manakin hybrid zone (Brumfield et al., 2001; Parchman et al., 2013), we expected little microsatellite differentiation between white-collared manakins and mainland hybrids, whereas we expected these two groups to show relatively high levels of genetic differentiation from golden-collared manakins. If island hybrids are genetically similar to mainland hybrids, then we expected little microsatellite differentiation between these groups, including sampled white-collared manakins.

We obtained genetic data from DNA collected from blood samples. We then performed a Bayesian clustering analysis in Structure version 2.3.4 (Pritchard et al., 2000), where we estimated the probability of the observed genotypes given a number of populations (K) ranging from one to six (the number of sampling sites plus two) by a Markov chain Monte Carlo (MCMC) method using a burn-in period of 10^5 iterations and 10^6 repetitions. We calculated the mean likelihood over 10 runs for each K and estimated the most likely number of clusters depending on the value of the ΔK statistic. The value of ΔK is based on the rate of change in the log probability of data between successive K values, as described in Evanno et al. (2005) using 'Structure Harvester' (Earl & VonHoldt, 2012). The K value with the highest associated ΔK was then used as prior information in 'Structure' to estimate q, the proportion of membership of each manakin genotype in each of the K clusters. Results were graphically visualized using Distruct version 1.1 (Rosenberg, 2004).

Behavioural Data

We recorded and analysed the jump-snap display behaviour from reproductively active adult male golden-collared manakins, white-collared manakins and hybrid manakins from Isla Bastimentos. As such, we collected data from golden-collared manakins around Gamboa, Panama (9°6′36″N, 79°41′24″W; $N = 120$ displays from 18 different males at six different leks, with an average of 5 ± 1.28 displays per male). We collected data from white-collared manakins around La Selva Biological station in Costa Rica (10°25′12″N, 84°W; $N = 61$ displays from eight different males at two different leks, with an average of 10.5 ± 2.62 displays per male). Finally, we collected data from hybrid manakins in the forests of Isla Bastimentos in the Province of Bocas del Toro, Panama (9°18′N, 82°7′48″W; $N = 26$ displays from five different males at two different leks, with an average of 6.4 ± 1.52 displays per male). Note that the locations where we sampled behaviour for golden-collared manakins and white-collared manakins differed from the locations where we collected DNA for genetic analyses (as described above); however, we collected behavioural data from the same hybrid population on Isla Bastimentos for which we had collected DNA samples.

Males typically perform a few jump-snap routines in each display bout before leaving the court; therefore, we defined a jump-

snap display as an uninterrupted series of jumps accompanied by wing-snaps within the court. We recorded behaviour with a high-speed video camera (MotionMeter, RedLake Inc., San Diego, CA, U.S.A.) that captured display movements at 125 frames/s. Given the buffering capacity of the high-speed camera, videography at this speed allowed us to collect 16 s of consecutive footage, which encompassed the duration of most jump-snap displays (average display length ≈ 10 s, maximum ≈ 16 s). We observed a colour-banded population of golden-collared manakins, whereas we identified both white-collared manakins and hybrid manakins solely by their display arenas constructed on the forest floor. Past work has indicated that males are faithful to these arenas for the duration of the breeding season and leave the arenas only for brief feeding bouts during the day. If other males approach the arena, the resident will aggressively displace the intruders. As a result, we could unambiguously identify arena owners based on their behaviour and their propensity to display at a particular site (McDonald et al., 2001).

For each male, a single recording session lasted ≈ 30 min and took place at a randomly chosen time between 0630 and 1600 hours. Each male in this study was observed for an equal amount of time. Observation sessions began by setting up the high-speed camera at a mean (± SD) distance of 5.12 ± 0.35 m from the edge of the focal male's arena. Observers sat ≈ 10 m away from this camera behind a camouflaged blind, and thus monitored the activity at the arena with binoculars. When focal males completed a single jump-snap display, the observer saved the video file so that it could be analysed later. Observers collected a mean (± SD) of 5.60 ± 3.38 distinct jump-snap routines per individual (range 1–13). Observers also noted during each jump-snap routine whether the male performed this display by himself, or whether a female joined the display in the so-called duo-dance. Because male manakins often alter their courtship displays in the presence of a female (Schaedler et al., 2021; Vanderbilt et al., 2015), including *Manacus* manakins (Barske et al., 2015), we incorporated this effect into our statistical analysis of the behaviour (see below).

We analysed slow-motion recordings of display behaviour with Observer Video Pro 4.0 (Noldus Information Technology, Wageningen, The Netherlands). As described by Barske et al. (2011), we quantified the following variables: (1) beard-up time, the time a male took to reach a stationary position with his beard exposed, once he landed on a sapling; (2) on-perch time, the time a male spent on a sapling between two consecutive jumps during a jump-snap routine; (3) wing-snap frequency, the total number of singular wing-snaps included in a fully sequenced jump-snap dance routine divided by the duration of the routine; (4) in-air time, the duration of a jump; (5) jump speed, the speed at which a male jumped from one sapling to another (computed as the distance between two saplings divided by the duration of the jump); (6) jump-snap duration, the total amount of time to perform a single jump-snap routine. These manoeuvres are illustrated visually in greater detail in Fig. 1 and in our Supplementary Video S1. Note that data obtained from recordings of golden-collared and white-collared manakin displays were partly used for previous publications (Barske et al., 2011, 2015).

We discarded observations where female presence was unknown ($N = 6$), and thus used a total of 221 observations in our models. Regarding individual response variables, we discarded one outlier for the beard-up behaviour and one outlier for the in-air behaviour, which were likely to be data entry mistakes.

Analyses below were run in R version 4.0.1 and RStudio version 1.3.959 (R Core Team, 2020). We used linear mixed models in 'lme4' (version 1.1–23) to investigate whether manakin species or female presence in the area impacted some of the male courtship parameters. Full models comprised male identity (ID) as random

factor, with population (i.e. study site), female presence and the interaction between these two variables as fixed factors. We created a different model for each of the six male behaviours of interest. For beard-up, in-air and on-perch times, we log-transformed the data to generate a more normal distribution. Otherwise, we used the 'check_model' function from the 'sjPlot' package (version 2.8.4) to verify that model assumptions were not violated (e.g. normality of residuals, normality of random effects, heteroscedasticity, homogeneity of variance and multicollinearity).

We used a full-null model comparison approach, where null models lacked the fixed factor of interest (i.e. population, female presence or interaction term). When full-null model comparisons were significant, we used the 'drop1' function with a likelihood ratio test ('stats' package, version 4.0.1, R Core Team, 2013) to extract *P* values for the factor of interest. We then ran Tukey post hoc pairwise comparisons using the 'emmeans' function (version 1.4.5; see Lenth et al., 2020) to investigate which species differed from the other. Finally, to investigate whether courtship parameters depended on male identity, we used the 'anova' function ('stats' package version 4.0.1; R Core Team, 2013) to compare full models described above and linear models lacking the male identity random effect. For full model details, see Appendix.

RESULTS

Genetics

We found evidence of strong genetic similarity between the mainland hybrid population and the Isla Bastimientos population, confirming that Bastimientos manakins were in fact all hybrids. Our population structure analysis revealed that the most probable number of clusters for interpreting the observed genotypes was $K = 2$, given the modal value of $\Delta K = 1263.03$ (Table 1). Therefore, we used two main partitions as prior population information for calculating the posterior probability of individual assignment. White-collared manakins and mainland hybrids from Tierra Oscura all showed high average proportions of individual genomes assigned to the first cluster ($q = 0.985$), whereas a significant average proportional membership of golden-collared manakins' genomes ($q = 0.986$) was recorded in a second distinct cluster. Importantly, manakins from the Island of Bastimentos were assigned to the same cluster of white-collared manakins and mainland hybrids with a high proportion of membership ($q = 0.993$; Fig. 2). This supports the view that the island birds are genetically similar to the mainland hybrids west of the genomic centre, which in turn are genetically more similar to white-collared manakins (Parchman et al., 2013; Parsons et al., 1993).

Table 1
Statistics based on the rate of change in the log probability of data between successive values of the possible number of populations K according to Evanno et al. (2005)

K	Mean Ln P(K) ± SD	Ln′ (K)	|Ln″ (K)|	ΔK
1	−1409.28 ± 0.04	–	–	–
2	−1208.08 ± 0.13	201.20	164.68	1263.03
3	−1171.56 ± 16.34	36.52	14.64	0.89
4	−1149.68 ± 2.77	21.88	15.440	5.55
5	−1143.24 ± 1.91	6.44	7.70	4.02
6	−1144.50 ± 4.72	−1.26	–	–

K: number of clusters (populations); mean Ln P(K): the model choice criterion based on the posterior probability of the data for a given K (Pritchard et al., 2000); Ln′ (K): the mean difference between successive likelihood values of K; \|Ln″(K)\|: the absolute value of the difference between successive values of L′(K); ΔK: mean of the absolute values of L″(K) averaged over 10 runs divided by the standard deviation of L(K).

Behaviour

Next, we compared display behaviour among the populations, noting that jump-snap dances were qualitatively similar among the two parental lineages and their hybrids. However, we did uncover strong evidence for population differences of select display components, particularly those that define the acrobatic nature of the *Manacus* dance and its associated motor challenge (Fig. 3). Beard-up duration varied significantly between the three groups ($\chi^2 = 26.77$, $P < 0.001$; Fig. 3a), with golden-collared manakins showing longer beard-up times than white-collared manakins ($P < 0.001$). Hybrid manakins more closely resembled golden-collared manakins ($P = 0.093$) and also showed significantly longer beard-up durations than white-collared manakins ($P = 0.039$). A female's presence did not affect beard-up times (female: $\chi^2 = 0.05$, $P = 0.80$; female * population: $\chi^2 = 4.50$, $P = 0.10$), and we did not uncover an effect of male identity on this display variable ($\chi^2 = 0.26$, $P = 0.60$).

Wing-snap frequency during the jump-snap routine also differed between populations ($\chi^2 = 16.08$, $P < 0.001$; Fig. 3b). Golden-collared manakins showed significantly lower wing-snap frequencies during the jump-snap dance, compared to white-collared manakins ($P < 0.001$). Hybrids were statistically indistinguishable from golden-collared manakins ($P = 0.74$), and thus also showed lower wing-snap frequencies than white-collared manakins ($P = 0.043$). Importantly, our model also revealed that these differences depended on whether a female was present, such that wing-snap frequencies on average across all populations increased in the presence of a female ($\chi^2 = 6.73$, $P < 0.001$). This effect was likely driven by the increase in wing-snap frequency of hybrids in the presence of a female, a finding further corroborated by a significant interaction term between female presence and population ($\chi^2 = 7.47$, $P = 0.020$). Indeed, post hoc analyses showed that, when a male displayed without a female, both golden-collared manakins and hybrids produced wing-snaps at frequencies that were far lower than those of white-collared manakins ($P \leq 0.005$). But, when males displayed alongside a female (duo-dance), then hybrids more closely resembled white-collared manakins, in that they

showed a nonsignificant tendency to increase their frequency of wing snapping relative to that of golden-collared manakins (post hoc P values: $P \geq 0.054$). This model also uncovered a significant effect of male identity ($\chi^2 = 11.28$, $P = 0.003$).

For the other behavioural elements of the jump-snap display, hybrids either closely resembled white-collared manakins or were intermediate relative to the two parental lineages (Fig. 4). For example, in-air times differed significantly between the three populations ($\chi^2 = 9.58$, $P < 0.001$; Fig. 4a), with golden-collared manakins showing shorter in-air times compared to hybrids ($P = 0.026$) and a tendency towards shorter in-air times compared to white-collared manakins ($P = 0.077$). These latter two populations (hybrids and white-collared manakins) were statistically indistinguishable from each other ($P = 0.70$). In-air times were unaffected by female presence (females: $\chi^2 = 1.75$, $P = 0.18$; female * population: $\chi^2 = 0.69$, $P = 0.70$), but we did find a significant random effect of male identity ($\chi^2 = 30.60$, $P < 0.001$).

There were clear population differences for on-perch times ($\chi^2 = 10.13$, $P = 0.006$; Fig. 4b). Golden-collared manakins showed longer on-perch times than white-collared manakins ($P = 0.013$), while hybrids showed no difference compared to either parental species ($0.21 \leq P \leq 0.73$). There was no effect of female presence (female: $\chi^2 = 1.19$, $P < 0.27$; female * population: $\chi^2 = 0.69$, $P = 0.70$), but again there was a significant random effect of male identity ($\chi^2 = 6.14$, $P = 0.04$).

Finally, we found no evidence of group differences for other behavioural elements of the dance. Jump-snap sequence duration, for instance, was indistinguishable among populations ($\chi^2 = 2.00$, $P = 0.36$; Fig. 4c), but we did uncover a significant effect of female presence ($\chi^2 = 24.17$, $P < 0.001$; female * population: $\chi^2 = 5.37$, $P = 0.060$). Indeed, consistent with past work (Barske et al., 2015), sequence duration was longer in the presence of a female (i.e. during the duo-dance) compared to when a male displayed alone. This model also showed a random effect of male identity ($\chi^2 = 24.86$, $P < 0.001$). Jump speed was also indistinguishable among the three populations ($\chi^2 = 4.20$, $P = 0.12$; Fig. 4d), with no effect of female ($\chi^2 = 0.56$, $P = 0.45$; female * population: $\chi^2 = 0.69$, $P = 0.70$) and only a significant random effect of male identity ($\chi^2 = 104.9$, $P < 0.001$).
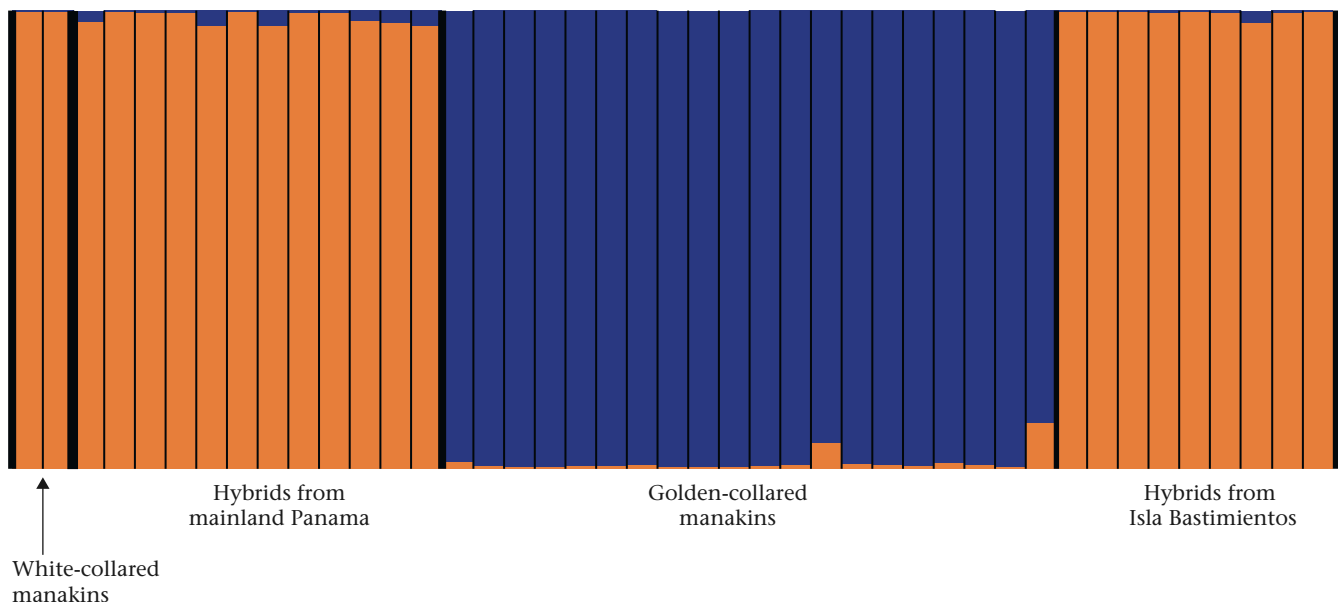

Figure 2. Plot of the proportions of manakin genome-wide ancestry belonging to each of the two clusters considered most likely to account for the observed *Manacus* genotypes. Each individual is represented by a vertical line partitioned into $K = 2$ segments (orange and blue), with lengths corresponding to the proportion of its genome originating from each of the two clusters inferred by a model-based Bayesian method (see Methods). Thick black lines separate the cluster of white-collared manakins and mainland hybrids, golden-collared manakins and hybrid birds sampled on Isla Bastimentos.
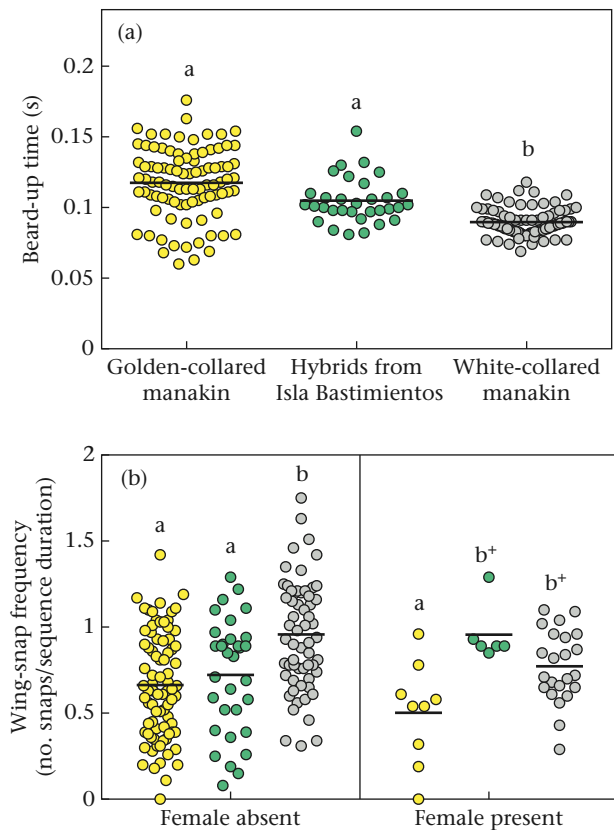
Figure 3. (a) Beard-up time (s) and (b) wing-snap frequencies during jump-snap routine from golden-collared manakins (yellow), white-collared manakins (grey), and their hybrids (green). Each dot represents single display bout (note that a single male can produce multiple displays, and thus we incorporated male identity into our statistical models as a random factor, see Methods). The horizontal black line in each distribution reflects the population average. Statistically significant ($P < 0.05$) differences among groups are denoted by differences in the letter atop each distribution. In (b), comparisons are separated by 'female absent' and 'female present' (horizontal axis) because our models showed a significant interaction term between female presence and population ($\chi^2 = 7.47$, $P = 0.02$). In the 'female present' groups, the cross (+) denotes marginally significant trends (i.e. golden-collared manakin versus hybrid, $P = 0.054$; golden-collared manakin versus white-collared manakin, $P = 0.068$; see Results). Note that the graph for 'female present' in (b) shows six different golden-collared manakin males, two different hybrid manakin males and seven different white-collared manakin males.

DISCUSSION

We studied variation in the elaborate courtship behaviour of golden-collared manakins, white-collared manakins and their hybrids (Table 2). In particular, we focused on a hybrid population from one of the small islands off the coast of Panama, confirming that the genetic background of these birds is similar to both mainland hybrids and their white-collared manakin parental species (Bennett et al., 2021; Whetmore, 1972). We then studied their courtship dance (jump-snap routine), which is performed around an arena on the forest floor cleared by the attendant male. Our results show that hybrid birds performed two acrobatic elements of this dance, beard-up time and wing-snap frequency, in a manner that closely resembled the golden-collared manakin parental line. Hybrids performed other elements of the display in a way that either did not differ from their white-collared manakin parental line (e.g. in-air time) or was intermediate to the two parental populations (e.g. on-perch time). Finally, some elements of the display were indistinguishable among populations entirely. Overall, while many of the differences we uncovered were undetectable to

the human eye, they likely could be perceived by the birds in question (Barske et al., 2011, 2015).

Why might hybrid manakins resemble golden-collared manakins, when the genetic background of these hybrids is more closely aligned with white-collared manakins (Brumfield et al., 2001; Parchman et al., 2013)? We hypothesize that the answer involves the introgression of discrete display elements into the hybrid population from the golden-collared parental line. Sexual selection likely underlies this process, as past work in golden-collared manakins showed that females prefer to mate with males that perform display manoeuvres, including beard-up times, at faster speeds (Barske et al., 2011). Indeed, we report here that beard-up times in hybrid birds closely resembled those of golden-collared manakins, which were slower to resume their statuesque posture, i.e. their beard-up times were longer, than white-collared manakins. This result may at first seem counterintuitive, because one might expect that hybrids would be more attractive if they performed displays at speeds that matched their white-collared parents. There are several possible explanations for this effect. For example, genes associated with yellow plumage, which might have introgressed into the hybrid population by sexual selection (Bennett et al., 2021; McDonald et al., 2001; Prado et al., 2009; Stein & Uy, 2006b), may also influence male performance by limiting the speed at which certain display manoeuvres can be performed. In other words, yellow plumage may be tied to a higher performance limit; thus, if such plumage introgresses into the hybrid population, so too would an elevated performance limit. This effect, in theory, would prevent males from generating certain display components (e.g. beard-up time) at faster speeds, even though females might prefer such behaviour when making mate choice decisions (Barske et al., 2011). Such coupling between plumage colour and beard-up behaviour seems especially likely, given that the latter presumably enhances a female's ability to evaluate the golden plumage of the manakin collar.

Our data on wing-snap frequency were more complicated in this regard. Male hybrids that performed dance routines alone produced lower wing-snap frequencies that resembled golden-collared manakins, whereas these same hybrids increased wing-snap frequency performance to match white-collared manakins when a female was present in the display. Our data are therefore consistent with the idea that this behavioural trait has introgressed into the hybrid population, but only in select display-related contexts. One possibility relates to the hypothesis outlined above, where an association between plumage and performance may constrain aspects of display output. Thus, introgression of plumage characteristics may alter the hybrids' physiology so that hybrids are more likely to produce wing-snaps during jump-snap behaviour like golden-collared manakins do. These same hybrids would then presumably increase wing-snap frequencies in the presence of a female, given that such behaviour is preferred. Inherent in this idea is that hybrids maintain a capacity for behavioural flexibility, whereas golden-collared manakins do not. If so, then the systems that set baseline physiological performance are likely under different genetic control than those that determine how individuals can deviate from these baselines. There are other possible explanations, including the idea that this part of the display has different functions in different contexts (female present versus female absent). Work in other species certainly shows that display function can vary according to the receiver who detects it (Loyau et al., 2005; Westcott, 1992; Zambre & Thaker, 2017) and/or the context in which it is performed (Gibson, 1996; Mateos & Carranza, 1999). Another possibility is that the hybrid population on Isla Bastimentos has a higher density of birds than on the mainland, creating a different competitive context that influences male display dynamics. Males perform their jump-snap displays both to court
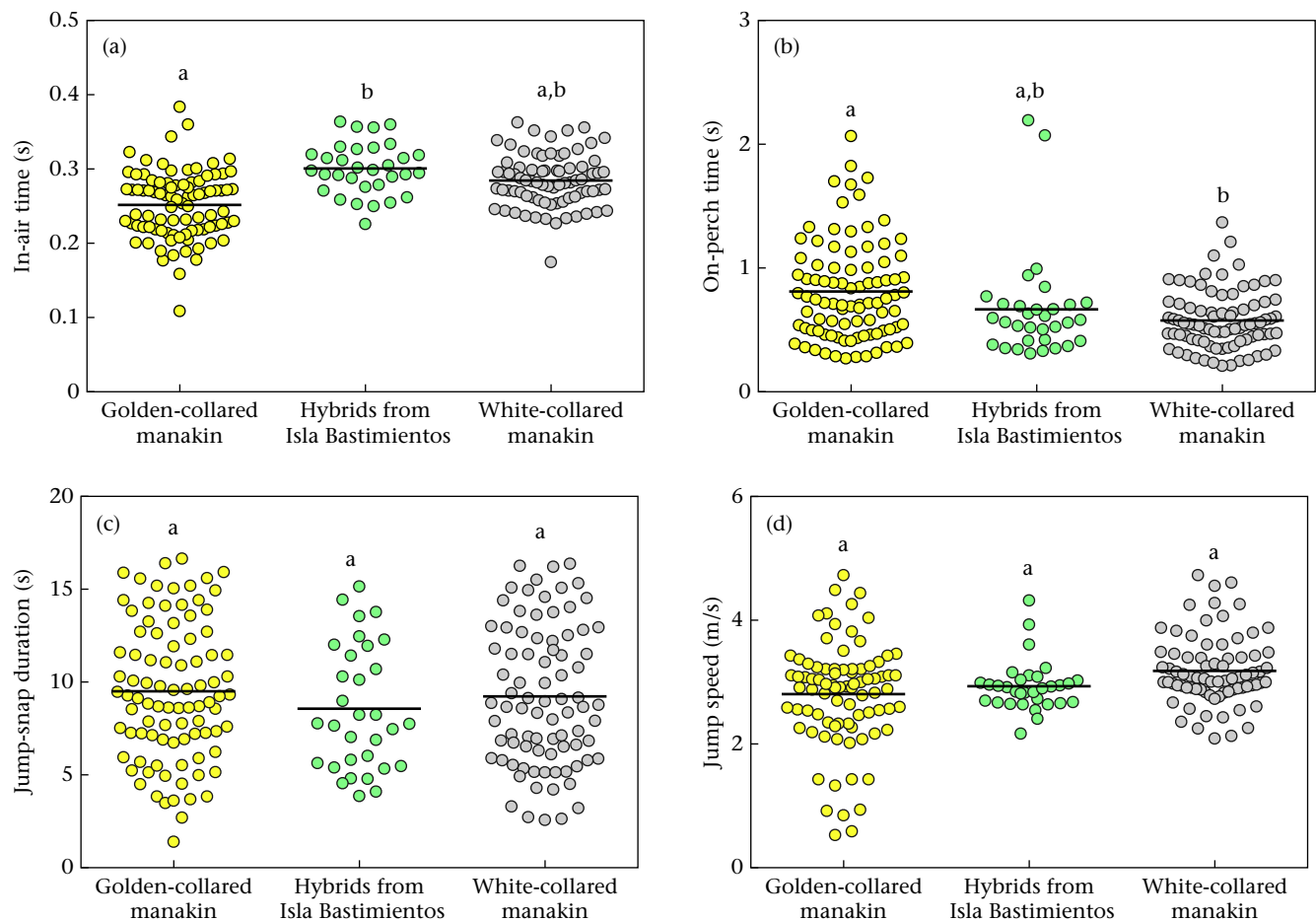
Figure 4. (a) In-air time (s), (b) on-perch time (s), (c) jump-snap duration (s) and (d) jump speed (m/s) from golden-collared manakins (yellow), white-collared manakins (grey) and their hybrids (green). Each dot represents a single display (note that a single male can produce multiple displays, and thus we incorporated male identity into our statistical models as a random factor; see Methods). The horizontal black line in each distribution reflects the population average. Statistically significant ($P < 0.05$) differences between groups are denoted by different letters above each distribution.

females and to compete with other males at the lek. Species differences in wing-snap performance may therefore arise through differential effects of inter- and intrasexual selection, with the latter force potentially favouring divergent agonistic tactics.

With these points in mind, we recognize that our data provide broad insight into the evolution of animal dance as a mechanism of sociosexual communication and advertisement. Species across much of the vertebrate tree of life use such behaviour to help court mates and compete with rivals (Fuxjager & Schlinger, 2015; Mitoyen et al., 2019; Tobiansky & Fuxjager, 2020), but our understanding of how these complex traits evolve remains in its infancy. Many studies have begun to suggest that such evolution occurs in a modular fashion (Hebets et al., 2016), whereby selection alters

discrete elements of a broader motor routine to fuel the diversification process (Miles & Fuxjager, 2018; Miles, Schuppe et al., 2018). Our current study supports this view, considering that we report population level differences in display behaviour only for discrete elements of the dance display. Had we observed evidence of population differences with respect to all components of dance, we might have concluded that this display evolves as a tightly integrated unit. However, because we uncovered a wide range of evolutionary signatures for different parts of the dance routine, we assume that many of the components can change independently. This view is consistent with other work in *Manacus* manakins; for example, divergence in the primary wing display of male bearded manakins, the roll-snap, occurs through species changes in speed

Table 2
Summary of behavioural results in golden-collared manakins, white-collared manakins and their hybrids

Behaviour	Summary of species differences	Hybrid phenotype?
Beard-up time[1]	Slower in golden-collared manakins	Golden-collared manakin dominant
Wing-snap frequency (count)[1]	Greater in white-collared manakins	Golden-collared manakin dominant in absence of female, white-collared dominant in presence of female
On-perch time[1]	Slower in golden-collared manakins	Intermediate between species
In-air time	Similar between species	Trending toward white-collared dominant
Jump-snap duration	Similar between species	Similar to both species
Jump speed	Similar between species	Similar to both species

[1] Denotes behavioural variables known to be associated with female preference in studies of golden-collared manakins (Barske et al., 2011).

(or snap frequency) independent of changes to display length (Miles, Goller et al., 2018).

Of course, when thinking about the evolution of courtship dancing, we must also acknowledge that variation in display routines among manakin populations may be linked to social feedback. Females in these populations may differ in their preferences for specific aspects of the display, with males adjusting their courtship during juvenile development accordingly. The role of learning in the establishment of choreographies and motor patterns has been scarcely investigated, with the exception of vocal learning (Spezie et al., 2022). Still, evidence of learning as a part of gestural display development is beginning to emerge in a range of species, adding a very intriguing layer to our understanding of animal dance displays and their evolution (Araya-Salas et al., 2019; Janisch et al., 2020; Schaedler et al., 2021; Schuppe et al., 2022).

There are a few important caveats to our study that are worth mentioning. The first is that our analyses were based on a relatively limited set of individuals (5 males) from the hybrid zone. While this sample size was sufficient to establish that discrete manoeuvres of the display differed between hybrid manakins and their parental taxa, a more thorough assessment of behaviour within the hybrid population would help us further describe how the elaborate dance displays of these birds evolve as modular display routines.

Another caveat is that we focused specifically on a hybrid population of *Manacus* that inhabits an island off the Coast of Panama. In theory, this means that behavioural differences between hybrids from the island and their parental lineages could be attributed to so-called 'island syndrome', or the evolution of phenotypic differences between island-dwelling individuals and their continental counterparts (Adler & Levins, 1994; Baeckens & Van Damme, 2020; Novosolov et al., 2013). We suspect, however, that such island effects do not strongly explain our results. First, the islands in the Bocas del Toro archipelago separated ≈5200 years ago (Anderson & Handley, 2002), and the specific island on which we sampled behaviour (Isla Bastimentos) is relatively close (≈1.3 km) to landmass that is well connected to the mainland hybrid populations. *Manacus* can fly extended distances over open waterways (Moore et al., 2008), suggesting that they might be able to travel between these locations, even if they do so infrequently. Second, we found no evidence of transgressive display behaviour, in which individuals from a hybrid population perform display elements that exceed the normal range of either parental population (Myers et al., 2022). This might be expected if differences in display routines in the hybrid population were largely due to effect of island syndrome. Finally, we did not see other key signatures of behavioural evolution that arise from island syndrome, including relaxed aggression and/or tameness (Baeckens & Van Damme, 2020). Still, we recognize that we cannot fully rule out effects of island life on the patterns of display behaviour that we document.

With the considerations described above in mind, future studies that explore how display traits of *Manacus* vary across the hybrid zone will be especially important going forward. Obtaining detailed behavioural data is substantially more time consuming and challenging than collecting information about plumage or genetic samples. Therefore, it will be important to carefully plan behavioural sampling across the hybrid zone based on our knowledge of morphological and genetic variation. Such research would have the power to test many of the idea we propose here, including whether our population differences truly reflect patterns of asymmetrical introgression of display traits by sexual selection. Furthermore, by coupling these studies to genetics research, we might gain additional insight into the molecular basis of elaborate display evolution and possibly even its role in supporting species boundaries. There is currently a rich literature identifying loci that are critical for performance-related behaviour that likely impacts gestural,

postural or dance displays (Mosher et al., 2007; Pearen et al., 2013; Wang et al., 2004).

Author Contributions

Julia Barske: conceptualization, methodology, data collection, writing − review and editing; **Matthew Fuxjager**: formal analysis, investigation, data curation, writing − original draft, visualization, funding acquisition; **Claudio Ciofi**: methodology, data collection, formal analysis, investigation, writing − original draft, visualization; **Chiara Natali**: formal analysis, investigation; **Barney Schlinger**: methodology, data collection, writing − review and editing; **Tim Billo**: methodology, data collection, writing − review and editing, funding acquisition; **Leonida Fusani**: conceptualization, methodology, data collection, formal analysis, investigation, data curation, writing − original draft, visualization, funding acquisition.

Data Availability

Data are available upon request.

Declaration of Interest

None.

Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2023.01.020.

References

Adler, G. H., & Levins, R. (1994). The island syndrome in rodent populations. *Quarterly Review of Biology, 69*(4), 473–490.

Anderson, R. P., & Handley, C. O. J. (2002). Dwarfism in insular sloths: Biogeography, selection, and evolutionary rate. *Evolution, 56*, 1045–1058.

Araya-Salas, M., Smith-Vidaurre, G., Mennill, D. J., González-Gómez, P. L., Cahill, J., & Wright, T. F. (2019). Social group signatures in hummingbird displays provide evidence of co-occurrence of vocal and visual learning. *Proceedings of the Royal Society B: Biological Sciences, 286*(1903). Article 20190666.

Baeckens, S., & Van Damme, R. (2020). The island syndrome. *Current Biology, 30*(8), R338–R339.

Baldassarre, D. T., & Webster, M. S. (2013). Experimental evidence that extra-pair mating drives asymmetrical introgression of a sexual trait. *Proceedings of the Royal Society B: Biological Sciences, 280*(1771). Article 20132175.

Baldassarre, D. T., White, T. A., Karubian, J., & Webster, M. S. (2014). Genomic and morphological analysis of a semipermeable avian hybrid zone suggests asymmetrical introgression of a sexual signal. *Evolution, 68*(9), 2644–2657.

Barske, J., Schlinger, B. A., & Fusani, L. (2015). The presence of a female influences courtship performance of male manakins. *Auk, 132*, 594–603.

Barske, J., Schlinger, B. A., Wikelski, M., & Fusani, L. (2011). Female choice for male motor skills. *Proceedings of the Royal Society B: Biological Sciences, 278*, 3523–3528.

Bennett, K. F. P., Lim, H. C., & Braun, M. J. (2021). Sexual selection and introgression in avian hybrid zones: Spotlight on *Manacus. Integrative and Comparative Biology, 61*, 1291–1309.

Billo, T. J. (2011). *Sexual selection, vocal signals, and plumage introgression in an avian hybrid zone* (Ph.D. thesis). University of Washington.

Brelsford, A., & Irwin, D. E. (2009). Incipient speciation despite little assortative mating: The yellow-rumped warbler hybrid zone. *Evolution: International Journal of Organic Evolution, 63*(12), 3050–3060.

Brumfield, R. T. (1999). *Evolution of brilliant male plumage traits in Manacus: Hybrid zones, molecular systematics, and riverine barriers* (Ph.D. thesis). College Park: University of Maryland.

Brumfield, R. T., Jernigan, R. W., McDonald, D. B., & Braun, M. J. (2001). Evolutionary implications of divergent clines in an avian (*Manacus*: Aves) hybrid zone. *Evolution, 55*(10), 2070–2087.

Butlin, R. K., & Neems, R. M. (1994). Hybrid zones and sexual selection. *Science, 265*(5168), 122.

Chapman, F. M. (1935). The courtship of Gould's manakin (*Manacus vitellinus vitellinus*) on Barro Colorado Island, Canal Zone. *Bulletin of the American Museum of Natural History, 68*, 472–521.

Cooney, C. R., Tobias, J. A., Weir, J. T., Botero, C. A., & Seddon, N. (2017). Sexual selection, speciation and constraints on geographical range overlap in birds. *Ecology Letters, 20*(7), 863–871.

Earl, D. A., & VonHoldt, B. M. (2012). Structure Harvester: A website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetics Resources, 4*(2), 359–361.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software Structure: A simulation study. *Molecular Ecology, 14*(8), 2611–2620.

Fusani, L., Barske, J., Day, L. D., Fuxjager, M. J., & Schlinger, B. A. (2014). Physiological control of elaborate male courtship: Female choice for neuromuscular systems. *Neuroscience & Biobehavioral Reviews, 46*, 534–546.

Fusani, L., Barske, J., Natali, C., Chelazzi, G., & Ciofi, C. (2018). Relatedness within and between leks of golden-collared manakin differ between sexes and age classes. *Behavioral Ecology, 29*(6), 1390–1401.

Fusani, L., Giordano, M., Day, L. B., & Schlinger, B. A. (2007). High-speed video analysis reveals individual variability in the courtship displays of male golden-collared manakins. *Ethology, 113*, 964–972.

Fuxjager, M. J., & Schlinger, B. A. (2015). Perspectives on the evolution of animal dancing: A case study in manakins. *Current Opinion in Behavioral Sciences, 6*, 7–12.

Gibson, R. M. (1996). Female choice in sage grouse: The roles of attraction and active comparison. *Behavioral Ecology and Sociobiology, 39*(1), 55–59.

Hebets, E. A., Barron, A. B., Balakrishnan, C. N., Hauber, M. E., Mason, P. H., & Hoke, K. L. (2016). A systems approach to animal communication. *Proceedings of the Royal Society B: Biological Sciences, 283*(1826). Article 20152889.

Higham, J. P., & Hebets, E. A. (2013). An introduction to multimodal communication. *Behavioral Ecology and Sociobiology, 67*(9), 1381–1388.

Hudson, E. J., & Price, T. D. (2014). Pervasive reinforcement and the role of sexual selection in biological speciation. *Journal of Heredity, 105*(S1), 821–833.

Janisch, J., Perinot, E., & Fusani, L. (2020). Behavioural flexibility in the courtship dance of golden-collared manakins, *Manacus vitellinus. Animal Behaviour, 166*, 61–71.

Janisch, J., Perinot, E., Fusani, L., & Quigey, C. (2021). Deciphering choreographies of elaborate courtship displays of golden-collared manakins using markerless motion capture. *Ethology, 127*, 550–562.

Kirschel, A. N., Blumstein, D. T., & Smith, T. B. (2009). Character displacement of song and morphology in African tinkerbirds. *Proceedings of the National Academy of Sciences of the United States of America, 106*(20), 8256–8261.

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2020). *Estimated marginal means, aka least-squares means*. https://github.com/rvlenth/emmeans.

Lill, A. (1974). Sexual behavior of the lek-forming white-bearded manakin (*Manacus manacus trinitatis* Hartert). *Zeitschrift für Tierpsychologie, 36*, 1–36.

Lipshutz, S. E., Meier, J. I., Derryberry, G. E., Miller, M. J., Seehausen, O., & Derryberry, E. P. (2019). Differential introgression of a female competitive trait in a hybrid zone between sex-role reversed species. *Evolution, 73*(2), 188–201.

Lipshutz, S. E., Overcast, I. A., Hickerson, M. J., Brumfield, R. T., & Derryberry, E. P. (2017). Behavioural response to song and genetic divergence in two subspecies of white-crowned sparrows (*Zonotrichia leucophrys*). *Molecular Ecology, 26*(11), 3011–3027.

Loyau, A., Jalme, M. S., & Sorci, G. (2005). Intra- and intersexual selection for multiple traits in the peacock (*Pavo cristatus*). *Ethology, 111*(9), 810–820.

Maan, M. E., & Seehausen, O. (2011). Ecology, sexual selection and speciation. *Ecology Letters, 14*(6), 591–602.

Mateos, C., & Carranza, J. (1999). Effects of male dominance and courtship display on female choice in the ring-necked pheasant. *Behavioral Ecology and Sociobiology, 45*(3), 235–244.

McDonald, D. B., Clay, R. P., Brumfield, R. T., & Braun, M. J. (2001). Sexual selection on plumage and behavior in an avian hybrid zone: Experimental tests of male–male interactions. *Evolution, 55*(7), 1443–1451.

McDonald, D. B., & Potts, W. K. (1994). Cooperative display and relatedness among males in a lek-mating bird. *Science, 266*(5187), 1030–1032.

Miles, M. C., & Fuxjager, M. J. (2018). Synergistic selection regimens drive the evolution of display complexity in birds of paradise. *Journal of Animal Ecology, 87*, 1149–1159.

Miles, M. C., Goller, F., & Fuxjager, M. J. (2018). Physiological constraint on acrobatic courtship behavior underlies a rapid sympatric speciation even in bearded manakins. *Elife, 7*. Article e40630.

Miles, M. C., Schuppe, E. R., Ligon, R. M., & Fuxjager, M. J. (2018). Macroevolutionary patterning of woodpecker drums reveals how sexual selection elaborates signals under constraint. *Proceedings of the Royal Society B: Biological Sciences, 285*. Article 20172628.

Mitoyen, C., Quigley, C., & Fusani, L. (2019). Evolution and function of multimodal courtship displays. *Ethology, 125*(8), 503–515.

Moore, R. P., Robinson, W. D., Lovette, I. J., & Robinson, T. R. (2008). Experimental evidence for extreme dispersal limitation in tropical forest birds. *Ecology Letters, 11*(9), 960–968.

Mosher, D. S., Quignon, P., Bustamante, C. D., Sutter, N. B., Mellersh, C. S., Parker, H. G., & Ostrander, E. A. (2007). A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genetics, 3*(5). Article e79.

Myers, B. M., Rankin, D. T., Burns, K. J., Brelsford, A., & Clark, C. J. (2022). *k*-mer analysis shows hybrid hummingbirds perform variable, transgressive courtship sequences. *Animal Behaviour, 186*, 67–84.

Novosolov, M., Raia, P., & Meiri, S. (2013). The island syndrome in lizards. *Global Ecology and Biogeography, 22*(2), 184–191.

Panhuis, T. M., Butlin, R., Zuk, M., & Tregenza, T. (2001). Sexual selection and speciation. *Trends in Ecology & Evolution, 16*(7), 364–371.

Parchman, T. L., Gompert, Z., Braun, M. J., Brumfield, R. T., McDonald, D. B., Uy, J. A. C., Zhang, G., Jarvis, E. D., Schlinger, B. A., & Buerkle, C. A. (2013). The genomic consequences of adaptive divergence and reproductive isolation between species of manakins. *Molecular Ecology, 22*(12), 3304–3317.

Parsons, T. J., Olson, S. L., & Braun, M. J. (1993). Unidirectional spread of secondary sexual plumage traits across an avian hybrid zone. *Science, 260*(5114), 1643–1646.

Pearen, M. A., Goode, J. M., Fitzsimmons, R. L., Eriksson, N. A., Thomas, G. P., Cowin, G. J., Wang, S. C. M., Tuong, Z. K., & Muscat, G. E. O. (2013). Transgenic muscle-specific Nor-1 expression regulates multiple pathways that effect adiposity, metabolism, and endurance. *Molecular Endocrinology, 27*(11), 1897–1917. https://doi.org/10.1210/me.2013-1205

Piertney, S. B., Shorey, L., & Höglund, J. (2002). Characterization of microsatellite DNA markers in the white-bearded manakin (*Manacus manacus*). *Molecular Ecology Notes, 2*(4), 504–505.

Prado, F., Billo, T., & Kerr, B. (2009). Introgression of sexually selected traits in lek-mating species. *Evolutionary Ecology Research, 11*, 1235–1250.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945–959.

R Core Team. (2013). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

R Core Team. (2020). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Ritchie, M. G. (2007). Sexual selection and speciation. *Annual Review of Ecology, Evolution, and Systematics, 38*, 79–102.

Rosenberg, N. A. (2004). Distruct: A program for the graphical display of population structure. *Molecular Ecology Notes, 4*(1), 137–138.

Sætre, G. P., Borge, T., Lindroos, K., Haavie, J., Sheldon, B. C., Primmer, C., & Syvänen, A. C. (2003). Sex chromosome evolution and speciation in *Ficedula* flycatchers. *Proceedings of the Royal Society of London, Series B: Biological Sciences, 270*(1510), 53–59.

Schaedler, L. M., Ribeiro, P. H. L., & Manica, L. T. (2021). Phenotype networks reveal differences between practice and courtship displays in swallow-tailed manakins. *Animal Behaviour, 171*, 29–39.

Schuppe, E. R., Cantin, L., Chakraborty, M., Biegler, M. T., Jarvis, E. R., Chen, C., Hara, E., Bertelsen, M. F., Witt, C. C., Jarvis, E. D., & Fuxjager, M. J. (2022). Forebrain nuclei linked to woodpecker territorial drum displays mirror those that enable vocal learning in songbirds. *PLoS Biology, 20*(9). Article e3001751.

Seddon, N. (2005). Ecological adaptation and species recognition drives vocal evolution in Neotropical suboscine birds. *Evolution, 59*(1), 200–215.

Shorey, L. (2002). Mating success on white-bearded manakin (*Manacus manacus*) leks: Male characteristics and relatedness. *Behavioral Ecology and Sociobiology, 52*, 451–457.

Spezie, G., Quigley, C., & Fusani, L. (2022). Learned components of courtship: A focus on postural displays, choreographies and construction abilities. *Advances in the Study of Behavior, 54*, 43–108.

Stein, A. C., & Uy, J. A. C. (2006a). Plumage brightness predicts male mating success in the lekking golden-collared manakin, *Manacus vitellinus. Behavioral Ecology, 17*(1), 41–47.

Stein, A. C., & Uy, J. A. C. (2006b). Unidirectional introgression of a sexually selected trait across an avian hybrid zone: A role for female choice? *Evolution, 60*, 1476–1485.

Tobiansky, D. J., & Fuxjager, M. J. (2020). Sex steroids as regulators of gestural communication. *Endocrinology, 161*(7). Article bqaa064.

Uy, J. A. C., & Stein, A. C. (2007). Variable visual habitats may influence the spread of colorful plumage across an avian hybrid zone. *Journal of Evolutionary Biology, 20*(5), 1847–1858.

Vanderbilt, C. C., Kelley, J. P., & DuVal, E. H. (2015). Variation in the performance of cross-contextual displays suggests selection on dual-male phenotypes in a lekking bird. *Animal Behaviour, 107*, 213–219.

Wang, Y. X., Zhang, C. L., Yu, R. T., Cho, H. K., Nelson, M. C., Bayuga-Ocampo, C. R., Ham, J., Kang, H., & Evans, R. M. (2004). Regulation of muscle fiber type and running endurance by PPARdelta. *PLoS Biology, 2*(10), 1532–1539.

West-Eberhard, M. J. (1983). Sexual selection, social competition, and speciation. *Quarterly Review of Biology, 58*(2), 155–183.

Westcott, D. (1992). Inter- and intra-sexual selection: The role of song in a lek mating system. *Animal Behaviour, 44*(4), 695–703.

Whetmore, A. (1972). *The birds of the Republic of Panama, Part 3. Smithsonian Miscellaneous Collections* (Vol. 150). Smithsonian Institution.

Yuri, T., Jernigan, R. W., Brumfield, R. T., Bhagabati, N. K., & Braun, M. J. (2009). The effect of marker choice on estimated levels of introgression across an avian (Pipridae: *Manacus*) hybrid zone. *Molecular Ecology, 18*(23), 4888–4903.

Zambre, A. M., & Thaker, M. (2017). Flamboyant sexual signals: Multiple messages for multiple receivers. *Animal Behaviour, 127*, 197–203.

Zonana, D. M., Gee, J. M., Bridge, E. S., Breed, M. D., & Doak, D. F. (2019). Assessing behavioral associations in a hybrid zone through social network analysis: Complex assortative behaviors structure associations in a hybrid quail population. *American Naturalist, 193*(6), 852–865.

Appendix

Table A1
Full model results for each behavioural variable tested among populations

	Number of parameters	AIC	BIC	Deviance	χ^2	df	P
Beard-up time							
Female presence	4	−60.572	−44.081	−70.572			
Simple model	6	−58.632	−38.842	−70.632	0.0599	1	0.8067
Species	4	−35.855	−22.662	−43.855			
Simple model	6	−58.632	−38.842	−70.632	26.777	2	1.532e-06
Simple model	6	−58.632	−38.842	−70.632			
Interaction model	8	−59.136	−32.749	−75.136	4.5034	2	0.1052
Male ID random effect variance		0.001902					
Male ID random effect SD		0.04361					
In-air time							
Female presence	4	−142.19	−128.99	−150.19			
Simple model	6	−147.77	−127.98	−159.77	9.581	2	0.008308
Species	4	−148.01	−131.52	−158.01			
Simple model	6	−147.77	−127.98	−159.77	1.7543	1	0.1853
Simple model	6	−147.77	−127.98	−159.77			
Interaction model	8	−144.46	−118.08	−160.46	0.6957	2	0.7062
Male ID random effect variance		0.01224					
Male ID random effect SD		0.1106					
On-perch time							
Female presence	4	253.0	269.49	243			
Simple model	6	253.80	273.59	241.80	1.199	1	0.2735
Species	4	259.93	273.12	251.93			
Simple model	6	253.80	273.59	241.80	10.131	2	0.00631
Simple model	6	253.80	273.59	241.80			
Interaction model	8	257.11	283.50	241.11	0.6918	2	0.7076
Male ID random effect variance		0.03095					
Male ID random effect SD		0.1759					
Jump-snap duration							
Female presence	4	1089.5	1106.0	1079.5			
Simple model	6	1067.3	1087.1	1055.3	24.179	1	8.779e-07
Species	4	1065.3	1078.5	1057.3			
Simple model	6	1067.3	1087.1	1055.3	2.0031	2	0.3673
Simple model	6	1067.3	1087.1	1055.3			
Interaction model	8	1065.9	1092.3	1049.9	5.3738	2	0.06809
Male ID random effect variance		1.213					
Male ID random effect SD		1.101					
Wing-snap frequency							
Female presence	4						
Simple model	6	97.276	117.07	85.276		1	
Species	4	109.358	122.55	101.358			
Simple model	6	97.276	117.07	85.276	16.082	2	0.0003219
Simple model	6	97.276	117.07	85.276			
Interaction model	8	93.798	120.19	77.798	7.4775	2	0.02378
Male ID random effect variance		0.01282					
Male ID random effect SD		0.1132					
Jump speed							
Female presence	4	302.19	318.50	292.19			
Simple model	6	303.63	323.21	291.63	0.5626	1	0.4532
Species	4	303.83	316.88	295.83			
Simple model	6	303.63	323.21	291.63	4.2042	2	0.1222
Simple model	6	303.63	323.21	291.63			
Interaction model	8	306.93	333.03	290.93	0.697	2	0.7058
Male ID random effect variance		0.4073					
Male ID random effect SD		0.6382					

Table A2
Estimates and variance parameters of the fixed effects for the simple models (containing female presence + species)

	Estimate	SE	t
Beard up			
(Intercept)	−2.261140	0.041251	−54.814
White-collared manakin	−0.130032	0.049344	−2.635
Golden-collared manakin	0.102294	0.047149	2.170
Female present	−0.006676	0.037679	−0.177
In air			
(Intercept)	−1.19588	0.05626	−21.257
White-collared manakin	−0.05692	0.07050	−0.807
Golden-collared manakin	−0.17698	0.06383	−2.773
Female present	−0.03856	0.02839	−1.358
On perch			
(Intercept)	−0.54168	0.11012	−4.919
White-collared manakin	−0.10271	0.13509	−0.760
Golden-collared manakin	0.21731	0.12541	1.733
Female present	0.09058	0.08081	1.121
Jump-snap duration			
(Intercept)	8.0521	0.7736	10.409
White-collared manakin	0.6053	0.9406	0.644
Golden-collared manakin	1.1461	0.8822	1.299
Female present	3.1899	0.6229	5.121
Wing-snap frequency (count)			
(Intercept)	0.72447	0.07858	9.220
White-collared manakin	0.24882	0.09685	2.569
Golden-collared manakin	−0.06621	0.08941	−0.740
Female present	−0.14649	0.05437	−2.694
Jump speed			
(Intercept)	2.92671	0.29576	9.896
White-collared manakin	0.29135	0.37513	0.777
Golden-collared manakin	−0.26686	0.33508	−0.796
Female present	0.06222	0.08321	0.748

Note that hybrids are the reference species in these models; thus, the predictors reflect the impact of a given level with respect to that reference (e.g. white-collared manakin versus hybrid as reference).

Table A3
Estimates for every level of the random effect Male ID (extracted with the 'ranef()' function, 'lme4' package)

	(Intercept)					
	Beard up	In air	On perch	Jump-snap duration	Wing-snap frequency (count)	Jump speed
ELA03	0.0053561847	0.120765343	−0.1457200581	−0.32245529	0.049803952	−0.609575196
ELA04	−0.0142731405	−0.097774976	−0.0001281738	−0.74421253	0.048649995	−1.216755641
ELA07	−0.0055629985	−0.090223612	−0.0700271980	0.76838025	0.081598782	0.648053960
ELA09	0.0107423662	0.054495817	0.1973325817	1.44967542	−0.092328805	−0.124325208
ELA10	0.0080607915	0.024965566	0.1253865928	0.74529243	−0.137077569	−1.460653738
ELA11	−0.0286410291	−0.228411000	−0.1875564881	−1.10041514	0.039557958	0.350149920
GOOSE12	0.0341111010	0.071299844	0.0486614865	0.88285290	−0.241759750	0.045928993
HIL01	0.0332239238	−0.099501772	0.1748658076	−0.48690802	−0.138915266	0.248263056
HIL03	0.0306364679	−0.068800573	0.2147572677	0.78229364	−0.088628458	0.202533582
HIL05	−0.0007989666	0.086990708	−0.1581972010	−0.57122069	0.102877720	0.460221253
OSCAR(A)	−0.0293106250	0.012853600	−0.0906063112	−0.44595796	0.171537783	0.003188005
OSCAR(B)	0.0083357438	−0.049913653	0.2316026917	0.53372450	−0.155313451	0.031812236
OSCAR(D)	−0.0078146318	0.011583932	−0.1097419152	−0.49412225	0.135884211	−0.057016771
OSCAR(E)	−0.0053215880	−0.045823722	−0.0799159518	−0.47649718	0.089651208	−0.023912462
RES01	−0.0019303254	−0.070897455	0.0292177827	−0.32912322	−0.029678568	0.333523487
RES02	−0.0027904011	−0.017249845	−0.2164880253	0.14371713	0.083929966	0.995127307
RES03	−0.0117259125	−0.076580353	−0.1125868576	−0.83728285	0.133954063	0.189503697
RES04	0.0227967535	0.107701803	−0.0683921718	−0.67231955	0.069185121	−0.447945874
RES05	−0.0140494182	0.026545799	0.0300092169	0.33834802	−0.005634016	−0.841150402
RES06	−0.0136150567	0.225437805	0.0324442946	−0.42337255	−0.082061607	−0.188054283
RLR03	−0.0038262158	−0.013523980	0.0386496737	0.83612369	0.025281130	0.287899354
RLR06	0.0230705860	−0.013448234	0.0836166195	0.12758750	−0.011068929	1.369321373
RLR08	−0.0366736092	0.129508959	0.0328163366	0.29589173	−0.049445469	−0.196136647
SAZ(A)	0.0250625192	−0.072934711	0.1422333993	0.90309224	−0.085034752	0.161124070
SAZ(B)	−0.0111919188	−0.014844618	0.0627840727	−0.04172398	−0.038675531	0.054926402
SAZ(C)	−0.0010356210	0.037029964	0.0012937213	0.18727853	−0.003811514	−0.182402986
SAZ(D)	−0.0189349162	−0.021231552	−0.1022119878	−0.21280533	−0.034518303	0.202465820
SHO(A)	0.0377416783	0.002076669	−0.0174104267	−0.63601214	0.041736081	−0.727275436
SHO(B)	−0.0308250174	−0.131283204	0.0669349973	0.96839452	0.031947374	−0.153162574
SHO(C)	−0.0024134836	0.155316827	−0.1444779786	−0.99223696	0.069126749	−0.272016612
SHO(D)	0.0015967594	0.045870625	−0.0091457976	−0.17598689	0.019229895	0.916341317