Differential introgression of a female competitive trait in a hybrid zone between sex-role reversed species

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Mating behavior between recently diverged species in secondary contact can impede or promote reproductive isolation. Traditionally, researchers focus on the importance of female mate choice and male–male competition in maintaining or eroding species barriers. Although female–female competition is widespread, little is known about its role in the speciation process. Here, we investigate a case of interspecific female competition and its influence on patterns of phenotypic and genetic introgression between species. We examine a hybrid zone between sex-role reversed, Neotropical shorebird species, the northern jacana (*Jacana spinosa*) and wattled jacana (*J. jacana*), in which female–female competition is a major determinant of reproductive success. Previous work found that females of the more aggressive and larger species, *J. spinosa,* disproportionately mother hybrid offspring, potentially by monopolizing breeding territories in sympatry with *J. jacana.* We find a cline shift of female body mass relative to the genetic center of the hybrid zone, consistent with asymmetric introgression of this competitive trait. We suggest that divergence in sexual characteristics between sex-role reversed females can influence patterns of gene flow upon secondary contact, similar to males in systems with more typical sex roles.

KEY WORDS: Asymmetric introgression, female competition, hybridization, jacana, sex-role reversal.

A classical goal of speciation research is to understand the factors that determine the maintenance or loss of species barriers, typically measured in sympatry after return from a period of allopatric divergence (Coyne and Orr 2004; Mallet 2008). Secondary contact between recently diverged lineages provides a natural laboratory for testing the establishment of reproductive isolation (Hewitt 1988), particularly for organisms that are not especially fecund or are difficult to breed in captivity. Traditionally, females have been considered the gatekeepers of species through avoiding costly mating with heterospecifics, whereas males are

expected to mate indiscriminately to maximize fitness (Darwin 1871; Bateman 1948; Andersson 1994). More recent research has highlighted the role of male–male competition in speciation (Seehausen and Schluter 2004; Tinghitella et al. 2018). However, little is known about the role *female–female competition* plays in the speciation process (Lipshutz 2017a), despite evidence that female–female competition over mating resources is widespread (Rosvall 2011; Cain and Ketterson 2012; Cain and Rosvall 2014).

Mating behavior can influence the likelihood of reproductive isolation between recently diverged species in secondary contact

(Irwin and Price 1999). For instance, competition over mates can either impede or promote hybridization between species, depending on its interaction with mate choice and the extent to which both forces select for similar or divergent trait optima (Qvarnström et al. 2012; Lipshutz 2017a; Tinghitella et al. 2018). Because sexual traits function both in competition for mating resources as well as mate attraction (Berglund et al. 1996; Hunt et al. 2009), their variation between species can have consequences for reproductive isolation. Sexual characteristics that function in competition include traits important in signaling dominance, such as coloration and vocalizations, morphological traits directly used in fighting, such as body size and weaponry, and behaviors, such as aggression and territoriality (Andersson 1994). Divergence in male traits important for competition can facilitate female-driven assortative mating (Dijkstra and Groothuis 2011; Okamoto and Grether 2013). Additionally, there is theoretical and empirical evidence that simultaneous sexual selection by male–male competition and female mate choice can facilitate or even cause speciation in sympatry if it is negative frequency dependent (van Doorn et al. 2004; Seehausen and Schluter 2004; Dijkstra et al. 2005). However, negative frequency-dependent selection may not be the most common outcome of trait-mediated competition. In fact, signals and traits involved in competitive interactions between sympatric species often converge to facilitate communication over territorial boundaries (Haavie et al. 2004; Tobias and Seddon 2009), which can promote hybridization (Qvarnström et al. 2010). Whether interspecific competition over mating resources erodes or maintains species barriers therefore depends on a variety of factors, including evolutionary history.

Sexual and natural selection can drive the introgression of alleles across a hybrid zone, resulting in the differential exchange of genes across semipermeable species boundaries (Harrison and Larson 2016). This introgression can be asymmetric, whereby traits and their associated genes transition from one population into another more extensively than in the other direction. Many examples of introgression are based on traits involved in local adaptation (Pardo-Diaz et al. 2012; Racimo et al. 2015; Lamichhaney et al. 2015; Bay and Ruegg 2017; Walsh et al. 2018). Traits involved in mate choice are typically thought to promote reproductive isolation, and are thus expected to have restricted introgression compared with ecological traits (Bridle et al. 2002). However, sexual selection has also been implicated in the introgression of traits across species barriers, for instance due to female preference and/or signal dominance of male traits (Parsons et al. 1993; Baldassarre et al. 2014). In one example–a hybrid zone between golden and white collared manakins (*Manacus candei* and *M. vitellinus*) in Panama—male differential aggression is hypothesized to explain the pattern of yellow plumage introgression across the hybrid zone relative to neutral genetic markers (Brumfield et al. 2001; McDonald et al. 2001). Morphological or behavioral

traits used directly in male competition for mates and/or territories can also introgress asymmetrically between lineages. In a hybrid zone between Italian and Western European lineages of the common wall lizard, head length along with coloration strongly predict male dominance and are asymmetrically introgressed relative to the genetic barrier between the lineages (While et al. 2015). As of yet, there are no studies demonstrating that female competition or male mate choice can facilitate introgression between hybridizing species. To understand the role of female competition in hybridization, a system in which female–female competition is a major determinant of mating success can help to generate predictions.

In the current study, we investigate the role of female competition in hybridization. Two species of Neotropical jacanas, the northern jacana (*Jacana spinosa)* and wattled jacana (*J. jacana),* are allopatric for most of their range but overlap in a narrow hybrid zone in Central America (Miller et al. 2014; Fig. 1). Jacanas are a classic example of a polyandrous mating system in that females defend harems containing multiple male mates (Jenni and Collier 1972; Emlen et al. 1998; Emlen and Wrege 2004a). Compared to males, females have larger body mass and show greater development of secondary sexual characters, including keratinous wing spurs (Emlen and Wrege 2004b). Body mass is a strong predictor of female mating success, and only the heaviest females control access to mates by defending breeding territories (Stephens 1984; Emlen and Wrege 2004b). Between the species, *J. spinosa* females have larger body mass, longer wing spurs and are more aggressive than *J. jacana* females; *J. spinosa* males also have longer wing spurs than *J. jacana* males, but they do not differ in body mass (Lipshutz 2017b). Previous work on the jacana hybrid zone found a bias in the introgression of mitochondrial DNA (mtDNA), in which phenotypic hybrids shared mtDNA haplotypes with *J. spinosa* but not *J. jacana* (Miller et al. 2014). One hypothesized explanation for why *J. spinosa* females disproportionately mother hybrid offspring is that they monopolize breeding territories and mates of both species in sympatry. As the role of female competition in the speciation process has been understudied relative to the role of male competition even for well-studied species in which females compete (Lipshutz 2017a), the *Jacana* hybrid zone presents an excellent opportunity to examine competition and hybridization from a female perspective.

Here, we test the hypothesis that female competition influences patterns of hybridization between *J. spinosa* and *J. jacana.* We first characterize the population structure of the hybrid zone using ~13,000 genome-wide SNPs to understand the extent of genomic divergence and hybridization between the species. We also compare the introgression of morphological traits associated with competition (body mass and wing spurs) relative to the transition of genome-wide loci and other phenotypic traits that differ between the species (facial ornamentation and dorsal plumage). We previously found that *J. spinosa* has a more competitive
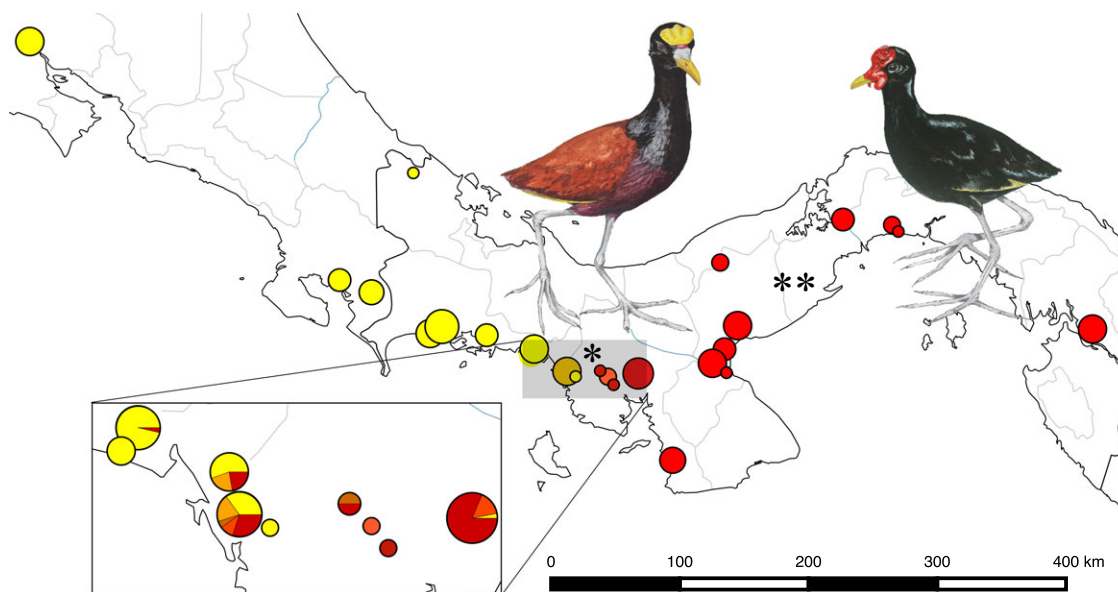
Figure 1. Genetic sampling map. Pie color represents proportion of *J. spinosa* parental (yellow), *J. spinosa* backcross (yellow-orange), F1 hybrid (orange), *J. jacana* backcross (red-orange), and *J. jacana* parental (red) for each locality based on STRUCTURE admixture proportions. Circle size represents number of individuals. * Indicates the genetic center of the hybrid zone. ** Indicates the female body mass center of the hybrid zone.

phenotype than *J. jacana,* and greater sexual size dimorphism (Lipshutz 2017b). We therefore predict that relative to other phenotypic traits and genome-wide markers, the geographic cline centers of female competitive traits will be shifted from the direction of *J. spinosa* into *J. jacana*–this would provide evidence of asymmetric introgression in the hybrid zone.

Methods
GENETIC SAMPLING
We sampled individuals from 28 localities across Costa Rica and Panama, focusing on the region of geographic overlap between *J. spinosa* and *J. jacana* in western Panama (Fig. 1; Table 1). This sampling expands upon Miller et al. (2014) to include more locations in the center of the hybrid zone and parental *J. spinosa* localities outside of the hybrid zone. In 2012 and 2014, we mist-netted and collected blood samples (40 μL) by brachial venipuncture from 186 individuals; we released birds after banding, ensuring that each sample was unique. We stored blood in Longmire's lysis buffer (Longmire et al. 1997) at ambient temperature. Eighty-five additional tissue samples from across the hybrid zone were provided as loans from the LSU Museum of Natural Science Collection of Genetic Resources (collected 1994–2004, $n = 18$) and the Smithsonian Tropical Research Institute Bird Collection (collected 2007–2012, $n = 67$; Supporting Information 1). We classified individuals as adults or immatures based on plumage (Jenni 1996) and sexed individuals in the field based on mass (Emlen and Wrege 2004b) or museum tag information. For

ambiguous cases we confirmed sex using PCR of the CHD regions of the W and Z chromosomes (Fridolfsson and Ellegren 1999, see Lipshutz 2017b for protocol details).

MORPHOLOGICAL MEASUREMENTS
For both sexes, we measured average left and right wing spur length from the middle of the curved base to the pointed tip with dial calipers to the nearest 0.1 mm, for both live and museum specimens. We measured body mass to the nearest 0.1 g with a Pesola scale or used information from the museum tag for the specimen associated with the LSU and STRI tissue loans. We included measurements from 51 additional birds sampled in 2015. We only included adults in our morphological and phenotypic datasets.

CLASSIFICATION OF PARENTAL AND HYBRID PHENOTYPES
We calculated a phenotypic hybrid index based on the diagnostic phenotypic characteristics of *J. jacana* and *J. spinosa* outside the hybrid zone (Fig. S1). We scored adult phenotypes on a scale of 0 (*J. jacana*) to 1 (*J. spinosa*) for each of three phenotypic traits: (i) dorsal plumage (black vs. brown), (ii) facial shield color and shape (red and bilobate vs. yellow and tri-lobate), and (iii) wattle size (presence of curved wattles vs. absence) from live birds ($n = 174$) and previously scored museum specimens ($n = 62$) from Miller *et al.* (2014). Two observers (T. Brown, S. Lipshutz) scored each trait independently and then averaged values per trait.

Table 1. Genetic and morphological sampling scheme and sizes for each locality.

Site	Locality	Latitude	Longitude	Distance	Q	Genetic N	Morphological N live	Morphological N museum	F_{IS}	Tajima's D	Pi
1	Palo Verde	10.344	−85.338	0	1	11	11	NA	0.09 ± 0.09	0.38 ± 1	$5.17^{-5} \pm 5.09^{-5}$
2	La Gamba	8.7	−83.194	298.15	0.995	8	8	NA	0.11 ± 0.07	0.17 ± 1.03	$5.33^{-5} \pm 5.25^{-5}$
3	Changuinola	9.4443	−82.6879	307.39	1	2	NA	1	NA	NA	NA
4	Coto 47	8.6	−82.967	324.75	1	10	6	NA	0.05 ± 0.1	0.25 ± 1.04	$5.31^{-5} \pm 5.23^{-5}$
5	La Barqueta	8.3061	−82.5746	378.5	0.997	10	12	NA	0.11 ± 0.06	0.17 ± 1.04	$5.18^{-5} \pm 5.2^{-5}$
6	Orillas del Rio	8.359	−82.527	379.67	0.997	23	22	6	0.15 ± 0.47	−0.34 ± 1.03	$3.71^{-5} \pm 4.64^{-5}$
7	Horconcitos	8.297	−82.167	416.23	0.998	7	3	2	0.09 ± 0.07	0.15 ± 1.02	$5.7^{-5} \pm 5.7^{-5}$
8	Las Lajas	8.176	−81.867	450.95	0.993	19	8	10	0.15 ± 0.62	−0.4 ± 0.9	$4.09^{-5} \pm 4.71^{-5}$
9	Remedios	8.214	−81.839	451.63	0.957	28	17	NA	0.23 ± 0.24	−0.39 ± 0.95	$3.69^{-5} \pm 4.62^{-5}$
10	Rio Tabasara	8.06	−81.64	479.32	0.673	11	NA	7	0.4 ± 0.32	0.17 ± 0.89	$7.08^{-5} \pm 6.69^{-5}$
11	Puerto Vidal	8.057	−81.617	481.64	0.602	21	5	6	0.37 ± 0.27	0.52 ± 0.91	$7.57^{-5} \pm 7.16^{-5}$
12	Jorones	8.0199	−81.5537	489.75	0.98	1	NA	1	NA	NA	NA
13	El Espino	8.0568	−81.375	504.42	0.081	1	NA	1	NA	NA	NA
14	Sona	8.02	−81.332	510.59	0.272	2	2	NA	NA	NA	NA
15	Tolica	7.9712	−81.2942	516.95	0.154	1	NA	1	NA	NA	NA
16	El Maranon	8.049	−81.115	523.47	0.09	32	24	NA	0.22 ± 0.09	0.46 ± 0.91	$8.26^{-5} \pm 7.78^{-5}$
17	La Corocita	8.0804	−81.0661	532.73	0.026	2	NA	2	NA	NA	NA
18	Coclecito	8.813	−80.5482	552.67	0	2	NA	2	NA	NA	NA
19	Las Macanas	8.115	−80.586	578.07	0.026	14	11	NA	0.18 ± 0.05	0.55 ± 0.9	$8.2^{-5} \pm 7.69^{-5}$
20	El Salado	8.2153	−80.5133	580.58	0.014	6	NA	5	0.23 ± 0.08	0.41 ± 0.91	$8.5^{-5} \pm 7.6^{-5}$
21	Puerto El Gago	8.3736	−80.4409	581.14	0.029	12	12	1	0.18 ± 0.05	0.51 ± 0.9	$8.3^{-5} \pm 7.75^{-5}$
22	Quebro	7.444	−80.882	584.93	0.049	12	8	NA	0.22 ± 0.12	0.5 ± 0.9	$7.87^{-5} \pm 7.32^{-5}$
23	Cenegon del Mangle	8.0502	−80.5001	589.73	0.015	2	7	2	NA	NA	NA
24	Gamboa	9.117	−79.693	634.22	0	17	11	5	0.21 ± 0.14	0.72 ± 0.89	$7.58^{-5} \pm 6.97^{-5}$
25	Tocumen	9.075	−79.3482	672.22	0	2	NA	1	NA	NA	NA
26	Pacora	9.0322	−79.3036	676.33	0	1	NA	NA	NA	NA	NA
27	Chepo	9.1664	−79.1124	695.45	NA	NA	7	NA	NA	NA	NA
28	Aruza Abajo	8.362	−77.9502	840.88	0	14	NA	9	0.22 ± 0.22	0.64 ± 0.88	$7.88^{-5} \pm 7.22^{-5}$

Q values, calculated from STRUCTURE admixture proportions for $K = 2$, indicate 1 for *J. spinosa* and 0 for *J. jacana*. Distance indicates geographic distance from western-most site, Palo Verde. Genetic N includes both live and museum samples as well as adults and juveniles, whereas juveniles were excluded from morphological samples. Population genomic summary statistics including fixation index (F_{is}), Tajima's D, and nucleotide diversity (Pi) ± standard deviation also included.

SEQUENCING

We extracted genomic DNA using a DNeasy® blood and tissue extraction kit following the manufacturer's recommended instructions (Qiagen, Valencia, CA). We sent DNA extracts to the Cornell University Biotechnology Resource Center in Ithaca, NY, USA, for genotyping-by sequencing (GBS). GBS reduced representation libraries were prepared and analyzed according to the methods of Elshire et al. (2011) using the restriction enzyme *Pst*I (CT-GCAG) for digestion and creating three libraries with 96 unique barcodes, one for each individual per plate. We sequenced 285 individuals on three lanes of the Illumina HiSeq 2500 platform, generating 4,281,246 merged 64 bp single-end sequence reads. Of these reads, 77.9% successfully mapped to a reference genome from *J. jacana* (E. Jarvis, unpubl. data) using BWA version 0.7.8–r455 (Li and Durbin 2009). The reference genome assembly was assessed for completeness using BUSCO (Benchmarking Universal Single-Copy Orthologs) (Simão et al. 2015) and was 80.7% complete relative to the lineage dataset aves_odb9 (Creation date: 2016-02-13, number of species: 40, number of BUSCOs: 4915). Single nucleotide polymorphisms (SNPs) were called using the Java program TASSEL-GBS 5.0 (Glaubitz et al. 2014). Filtering in the pipeline included minor allele frequency >0.01 and missing data per site $<10\%$, resulting in 360,689 variants for 271 individuals. Mean individual depth was 8.2 and mean site depth was 6.4. For population structure analyses we further filtered our SNP dataset in VCFtools v.4.2 (Danecek et al. 2011.) and using custom scripts (https://github.com/joanam/scripts) to include minor allele frequency >0.05 and linkage disequilibrium <0.1, resulting in 13,339 genome-wide biallelic SNPs for the 271 individuals. Sequence data are available in the Short Read Archive under BioProject PRJNA494934 and BioSamples SAMN10185940-SAMN10186205.

CLASSIFICATION OF PARENTAL AND HYBRID GENOTYPES

To visualize clustering of parental and hybrid genotypes, we conducted a genetic principal components analysis (PCA) using the 13,339-SNP dataset for all individuals with the package SNPRelate (Zheng et al. 2012) in R (R-Core-Team 2015). We also inferred individual assignment to species using the Bayesian clustering program STRUCTURE (Pritchard et al. 2000). We limited our estimation of assignment to two populations ($K = 2$) for *J. spinosa* and *J. jacana*. For 10 replicates of $K = 2$ we used a burn-in period of 100,000 followed by 500,000 Monte Carlo iterations, and an admixture model with correlated allele frequencies. We used CLUMPP (Jakobsson and Rosenberg 2007) to account for potential multimodality and label switching among replicates and Distruct2.py (Rosenberg 2004; Raj et al. 2014) to visualize admixture proportions (Q). We classified individuals as hybrids if $0.1 < Q < 0.9$. We further classified genotypes as parental *J.*

spinosa or *J. jacana,* F1 hybrid, or backcross in the package HIest in R, which estimates genotypes based on heterozygosity and the proportion of alleles from parental populations (Fitzpatrick 2012). For this dataset we used VCFtools to identify 344 diagnostic SNPs that were fixed between allopatric populations of *J. spinosa* and *J. jacana* and had no missing data.

To estimate genome-wide differentiation between the species, we used VCFtools to calculate Weir and Cockerham weighted F_{ST} between allopatric parental individuals, excluding localities with admixed individuals based on STRUCTURE. We also examined patterns of isolation by distance within each species using a Mantel test in the R package vegan (Oksanen et al. 2017), again excluding localities with admixed individuals. We calculated pairwise F_{ST} within each species across sampling localities with six individuals or more (Table 1) in VCFtools and calculated pairwise geographic distances from GPS coordinates using the "earthdist" function in the R package fossil (Vavrek 2011). We based our calculation of isolation by distance following Rousset (1997) with log(distance) and Fst/(1-Fst).

GEOGRAPHIC CLINE ANALYSES

Examining clinal transitions (i.e., gradual changes in traits or allele frequencies across a geographic range) between species for traits under selection relative to neutral genetic transitions between species can provide insight into their role in maintaining species barriers (Harrison and Larson 2014). Clines for traits that transition narrowly relative to dispersal ability are expected to contribute to reproductive isolation and traits that move freely are reputed to be neutral, whereas genetic loci that introgress more than neutral loci indicate selection favoring introgression, for example adaptive introgression (Barton 1979; Barton and Hewitt 1985).

To estimate the extent of introgression between *J. spinosa* and *J. jacana* across the hybrid zone, we fit genomic and morphological data to geographic cline models (Barton and Hewitt 1985) using the Metropolis–Hastings Markov chain Monte Carlo algorithm in the R package HZAR (Derryberry et al. 2014). To evaluate the genetic center and width (1/maximum slope) of the hybrid zone, we estimated clinal transitions of Q from STRUCTURE for $K = 2$ for all genetic samples collected from 2010 to 2014. For the Q cline we fixed the variance and the mean at either end of the cline to 0 (eastern-most *J. jacana*) or 1 (western-most *J. spinosa*) and evaluated three cline models with none (model 1), mirrored (model 4), or both tails separately (model 5). These tails are exponential decay curves with the parameters delta, indicating distance from the cline center to the tail, and tau, tail slope. Additionally, we estimated clines for genetic loci that were diagnostic between the species, meaning they had an allele frequency >0.9 on one end of the cline and <0.1 on the other end. We filtered

out loci with excess heterozygosity using a cutoff of $P = 0.05$ in VCFtools.

We also estimated clines for competitive traits, including wing spur length and body mass, as well as phenotypic traits that were diagnostic between the species, including phenotypic hybrid index and its individual components dorsal plumage and facial shield index. We combined data from both sexes for these traits, since jacanas are sexually monomorphic in plumage coloration and ornamentation shape (Emlen and Wrege 2004b). In contrast, all competitive traits, including body mass and average spur length, were sexually dimorphic (Lipshutz 2017b), so we estimated clines separately for males and females for these traits. For all clinal analyses we included all live and museum samples collected from 2010 to 2014, but did not include earlier museum specimens. We fit phenotypic traits to three standard cline models: one with fixed scaling and no tails (model 1), one with free scaling and no tails (model 2), and one with free scaling and both tails (model 3) (Derryberry et al. 2014).

Patterns of asymmetric introgression are often visualized by cline centers for traits under directional selection shifted relative to the neutral genetic transition between species (Brumfield et al. 2001). To assess whether any two given clines were coincident (i.e., overlap in their centers), we compared their range of two-log likelihood support for estimated center values (hereafter CIs). We followed the same procedure to assess concordance, that is whether cline widths were equal.

Results

POPULATION STRUCTURE AND CLASSIFICATION OF PARENTAL AND HYBRID GENOTYPES

J. spinosa and J. jacana are associated with distinct genomic clusters and hybridize

In the genetic PCA of 13,339 SNPs, the first eigenvalue (hereafter genetic PC1) explained 51.8% of variation in the data (Fig. 2). Subsequent eigenvalues explained much smaller percentages of variation (PC2 = 2.3, PC3 = 1.7, PC4 = 1.7, etc.). Both genetic PC1 and genome-wide admixture proportions (Q) for $K = 2$ distinguished the parental species and hybrids across the hybrid zone (Figs. 1, 2) and were strongly correlated ($r = 0.99$, $P < 2.2 \times 10^{-16}$, Fig. S2). STRUCTURE analyses demonstrated that all individuals sampled from localities 1–8 had a high probability of belonging to *J. spinosa* (Q > 0.9, mean = 0.985 ± SD 0.11), and all individuals from localities 17–20 and 23–28 had a high probability of belonging to *J. jacana* (Q < 0.1, mean = 0.001 ± 0.003) (Fig. 1, Table 1). Eighteen hybrid individuals (0.1 < Q < 0.9) were sampled at seven different localities, spanning 105 km in the direction of the major geographical axis of the hybrid zone. The presences of individuals with Q > 0.9 and Q < 0.1 were found in sympatry at five localities, spanning 72 km (Fig. 1, Table 1).

Genome-wide differentiation between *J. spinosa* and *J. jacana* in their allopatric populations, estimated using Weir and Cockerham weighted F_{ST}, was 0.52. Within *J. spinosa,* pairwise weighted F_{ST} ranged from 0 to 0.03 between sampling localities, with the exception of the comparison between Palo Verde and Coto 47, which was 0.05. For *J. jacana,* pairwise weighted F_{ST} ranged from 0.01 to 0.03. We found that *J. jacana* had a pattern of isolation by distance ($r = 0.86$, $P = 0.001$), but *J. spinosa* did not ($r = 0.46$, $P = 0.057$) (Fig. S3).

Classification of parental and hybrid genotypes based on heterozygosity and the proportion of alleles from parental populations indicated both F1 hybrids and backcrosses with *J. spinosa* and *J. jacana,* but no F2 hybrids (Fig. 3). Accounting for the male-biased sex ratio typically found in jacanas (Emlen and Wrege 2004b), we did not find that F1 hybrids were more commonly male than female ($X^2 = 0.51$, $P = 0.47$). Hybrid phenotypes are intermediate to both species in facial shield shape, wattle length, wing spur length, and dorsal plumage coloration (Fig. S1).

GEOGRAPHIC CLINAL TRANSITIONS

Female body mass is asymmetrically introgressed relative to genome-wide markers

The best-supported models for each trait are reported in Table 2. For Q (genome-wide admixture proportions), the cline center was estimated near Puerto Vidal at 483.2 km (CI: 479.2–489.3) from the western-most site, with a width of 32.4 km (CI: 27.5–41.8). For female body mass, the cline center was estimated farther east at 611.0 km (CI: 550.9–672.9), near Gamboa. The log-likelihood values for the female body mass cline center do not overlap with those of the Q cline center, indicating that larger female body mass associated with *J. spinosa* is asymmetrically introgressed relative to genome-wide markers (Fig. 4). Another way to interpret these results is to compare female body mass in sympatry and allopatry. Near the hybrid zone genetic center, females with *J. jacana* genotypes have an average body mass more similar to *J. spinosa* (*J. jacana* = 157.7 g, *J. spinosa* = 167.0 g), and the species do not significantly differ in female body mass (Welch Two Sample *t*-test: $t = -1.41$, df = 35.9, $P = 0.17$). In allopatry, however, female *J. jacana* body mass (134.3 g) is significantly smaller than female *J. spinosa* body mass (169.7 g) ($t = -5.72$, df = 22.35, $P = 8.94 \times 10^{-6}$). Plotting individual variation in female body mass across the hybrid zone for each species (Fig. S4) reveals that *J. jacana* female body mass significantly increases in sympatry ($R^2 = 0.17$, $P = 0.0043$). The cline width of female body mass was estimated at 288.5 km (CI: 140.4–485.1), and its log likelihood values also do not overlap with Q cline width. Male body mass did not differ between the species, so we did not fit cline models for this trait.

We identified 8479 genome-wide loci that were diagnostic and clinal between the species (Fig. 5). The median cline center

Table 2. Best-supported model and parameters for cline center, width, and shape of genotypic and phenotypic clinal transitions.

Model information	Q value	Body mass	Wing spur length	Wing spur length	Facial shield index	Dorsal plumage	Phenotypic hybrid index
Sex	Both	Female	Female	Male	Both	Both	Both
Best model	Model 5	Model 2	Model 2	Model 2	Model 3	Model 3	Model 2
Center	483.2 (479.2–489.3)	611.0 (550.9–672.9)	501.9 (434.3–560.1)	525.2 (367.3–863.6)	474.8 (473.5–476.0)	484.7 (482.0–489.5)	476.7 (473.6–480.2)
Coincident with Q center?	—	No	Yes	Yes	No	Yes	Yes
Width	32.4 (27.5–41.8)	288.5 (140.4–485.1)	359.9 (0.1–580.8)	148.9 (3.96–915.3)	34.3 (31.1–36.7)	39.5 (30.3–46.6)	29.3 (19.8–36.1)
Concordant with Q width?	—	No	Yes	Yes	Yes	Yes	Yes
muL	0	171.5 (164.7–178.4)	15.23 (12.96–16.91)	9.28 (8.94–10.16)	1 (0.98–1)	0.99 (0.97–1)	0.03 (0.02–0.04)
muR	1	128.5 (116.1–136.7)	8.69 (7.49–11.11)	7.91 (4.5–8.61)	0.02 (0.01–0.02)	0.01 (0.01–0.02)	0.99 (0.98–0.99)
varL	0	241.4 (115.1–436.9)	6.03 (3.11–13.57)	3.14 (1.87–4.44)	0	0	0
varR	0	0.11 (0–179.0)	0.24 (0–7.14)	1.46 (0.08–2.63)	0	0	0
varH	0.21 (0.14–0.3)	259.4 (34.2–606.0)	4.58 (0.1–19711.9)	0 (0–11.81)	0.41 (0.23–0.46)	0.14 (0.09–0.22)	0.24 (0.12–2.01)
deltaL	69.6 (59.8–90.0)	None	None	None	2.7 (2.0–6.6)	30.05 (21.4–34.2)	None
deltaR	1.41 (0–16.2)	None	None	None	128.72 (64.4–288.1)	37.5 (64.4–288.1)	None
tauL	0.18 (0.14–0.25)	None	None	None	0.48 (0.4–0.5)	0.09 (0.4–0.48)	None
tauR	0.44 (0.37–0.59)	None	None	None	0.25 (0.21–0.93)	0.28 (0.21–0.93)	None

Model 1 has fixed scaling (mean fixed at 0 or 1 and variance fixed at 0 for either end of the cline). Model 2 has free scaling and no tails. Model 3 has free scaling and both tails. Model 4 has fixed scaling and mirrored tails. Model 5 has fixed scaling and both tails. Mu represents the mean allele frequencies and phenotypic trait values for the left and right cline tails. Delta and tau are exponential decay curve parameters for left and right tails.
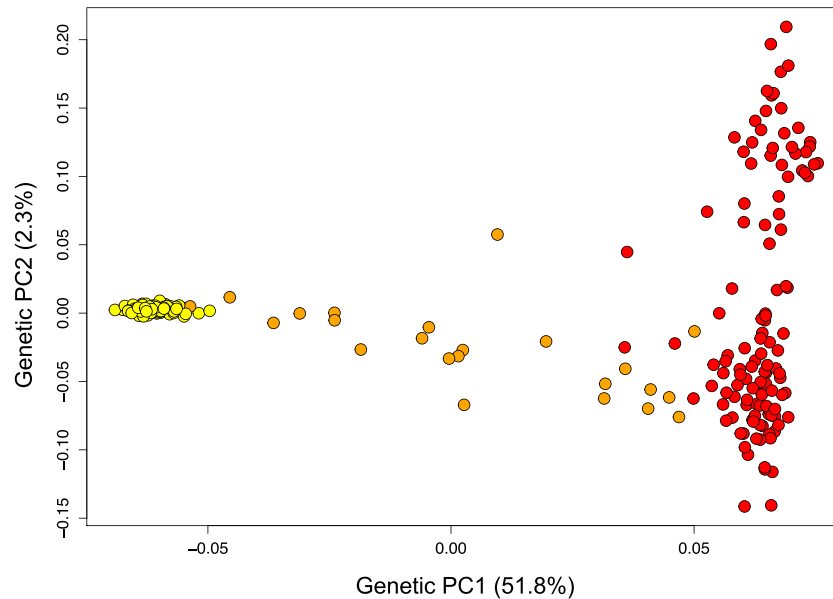
Figure 2. Genetic PC1 and PC2 of 13,339 genome-wide SNPs. Color codes indicate phenotypic classification based on STRUCTURE admixture proportions for $K = 2$: yellow = *J. spinosa* (Q > 0.9), orange = hybrid (0.1 < Q < 0.9), red = *J. jacana* (Q < 0.1).
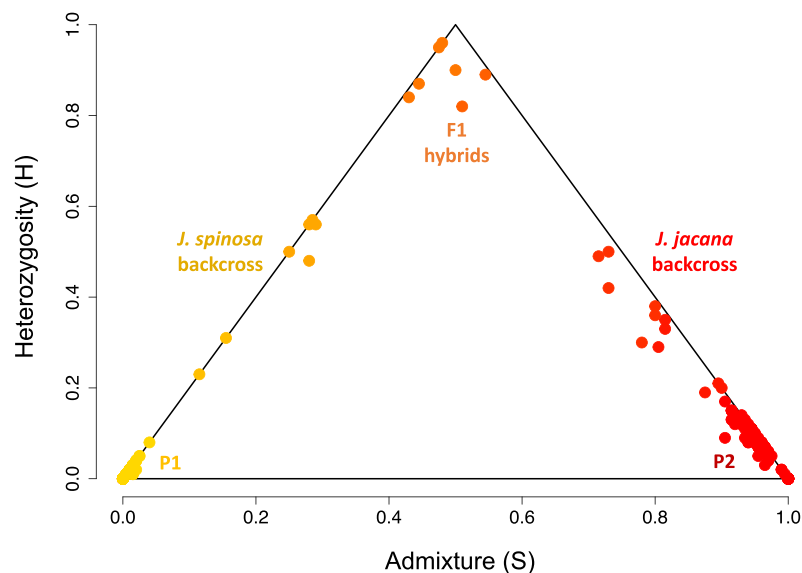

Figure 3. Classification of hybrid and parental genotypes indicated the presence of hybrid F1s (orange) as well as *J. spinosa* backcrosses (left side center) and *J. jacana* backcrosses (right side center).

of these diagnostic loci was 490.1 km from the western-most site, and 43% of these cline centers fell within the narrow confidence interval of the Q cline center (Fig. S5). In contrast, less than 1% (23) of these diagnostic loci had cline centers within the wider confidence interval of the female body mass cline center.

Other competitive traits are not asymmetrically introgressed in the hybrid zone

For female wing spur length, the cline center was estimated at 501.9 km (CI: 434.3–560.1), slightly shifted to the east of the Q

cline center (Fig. 6; Table 2). However, the log-likelihood values overlap with Q cline center, suggesting that the female wing spur and Q cline centers are coincident. The cline width for female wing spur length was estimated at 359.9 (CI: 0.1–580.8). The overlap of these log-likelihood values with Q cline width suggests they are concordant.

For male wing spur length, the cline center was estimated at 525.16 km (CI: 367.3–863.6), also shifted east of the Q cline center. However, the log-likelihood values overlap with Q cline center, suggesting that the male wing spur cline and Q cline centers
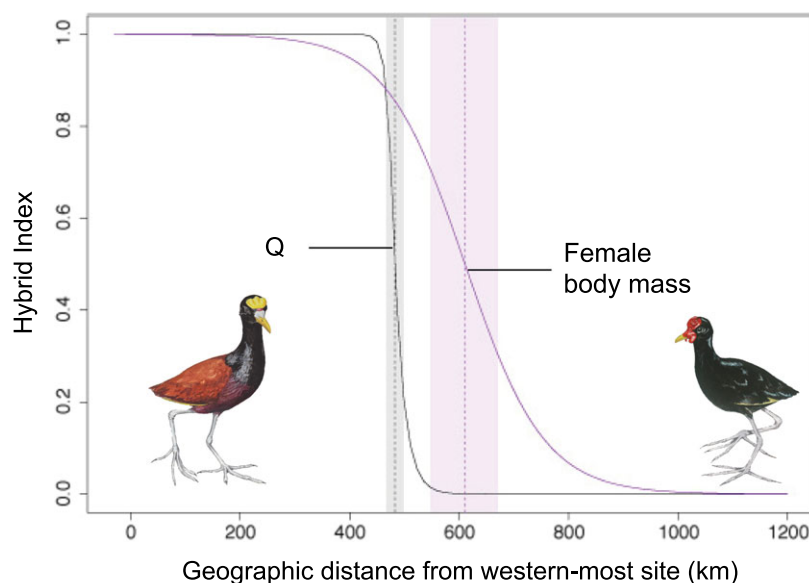
Figure 4. Best-supported cline models for Q (based on STRUCTURE admixture proportions for $K = 2$ from 13,339 genome-wide SNPs) and female body mass. Dotted lines indicate cline centers, and colored vertical bars indicate confidence intervals. Distance depicted from western-most *J. spinosa* locality in Palo Verde, Costa Rica.
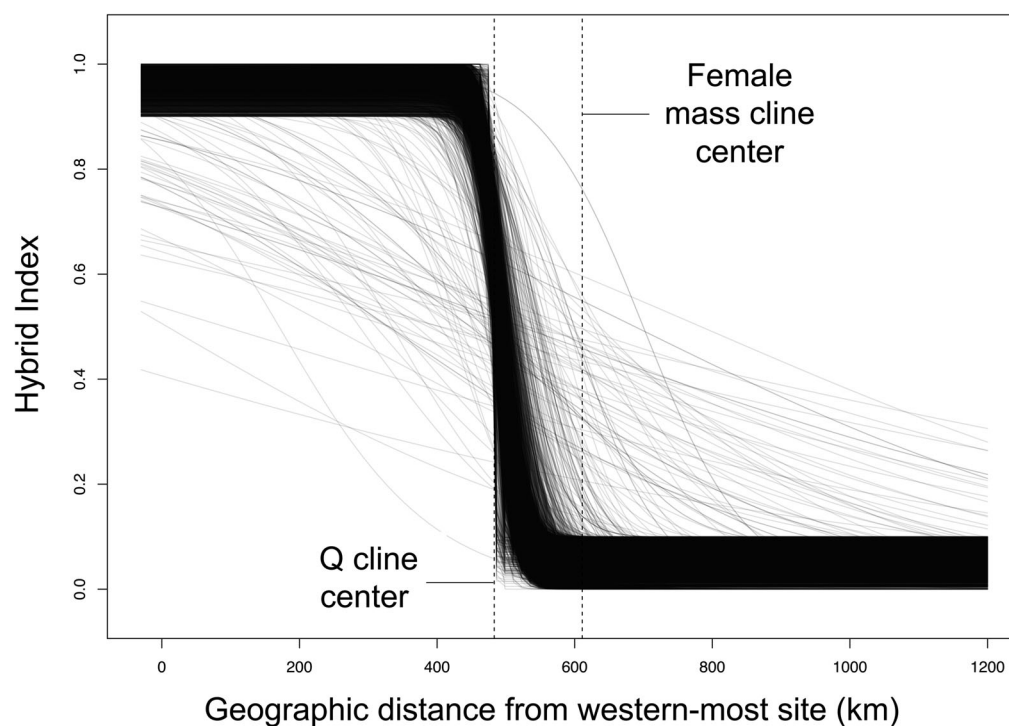

Figure 5. Best-supported cline models for diagnostic genomic loci. Geographic cline models for 8479 diagnostic, genome-wide loci in Hardy–Weinberg equilibrium.

are coincident. The cline width for male wing spur was estimated at 148.9 km (CI: 4.0–915.3). This overlaps with the log-likelihood values for Q cline width and spans the entire hybrid zone, suggesting the cline widths are not independent.

Facial ornamentation and plumage are concordant with neutral genetic markers

In contrast with female body mass, the cline widths of species-specific traits including facial shield index (34.3 km, CI: 31.1–36.7), dorsal plumage index (39.5 km, CI: 30.3–46.6), and
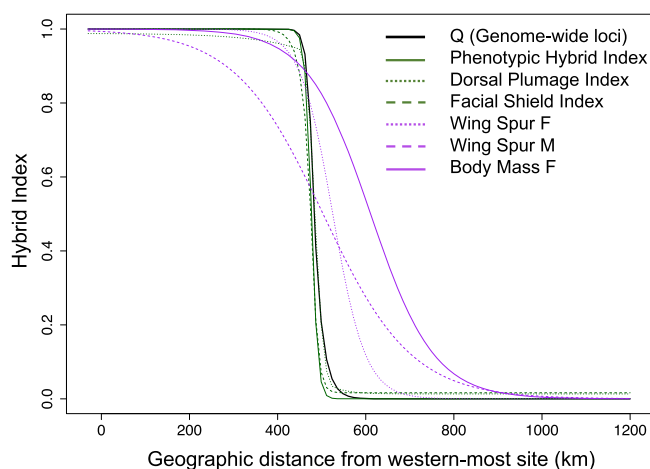
Figure 6. Best-supported cline models for Q (black, genome-wide loci from STRUCTURE for $K = 2$), competitive traits (purple) and putative species recognition traits (green).

phenotypic hybrid index (29.3 km, CI: 19.8–36.1), were concordant with Q cline width (Fig. 6, Table 2). However, the cline center for facial shield index was slightly west of the Q cline center. The models for wattle index had low likelihoods and were excluded from analyses.

Discussion

DIFFERENTIAL INTROGRESSION OF FEMALE BODY MASS, A SEXUALLY SELECTED COMPETITIVE TRAIT

We found that a female competitive trait–body mass–has a cline center shifted east relative to genome-wide markers and other species-specific traits tested. This pattern is suggestive of asymmetric introgression from the larger *J. spinosa* into *J. jacana* across the jacana hybrid zone. Considering that female *J. spinosa* are larger in morphological traits used in competition and more aggressive than female *J. jacana* (Lipshutz 2017b), our results are consistent with the hypothesis that female competition influences patterns of introgression between the jacana species. Our findings align with studies of other hybrid zones (Baldassarre et al. 2014; Yang et al. 2018) in which traits under selection by either female choice or male competition introgress asymmetrically relative to the genome-wide cline center between species.

Interspecific competition may select for increased female *J. jacana* body mass in sympatry, though the underlying mechanism has not yet been determined. The cline shift of female body mass across the hybrid zone could be driven by the adaptive introgression of *J. spinosa* alleles for larger body mass into *J. jacana*. Loci controlling variation in avian body mass have been identified on several chromosomes (Henriksen et al. 2016), and given that size is typically a quantitative trait with small effect loci on many different chromosomes, it is possible that introgression has

contributed the underlying genetic variation targeted by selection. Future work should identify whether such loci in jacanas have asymmetrically introgressed across the hybrid zone relative to the genome-wide average. An alternative hypothesis is that there is increased selection on existing genetic variation for larger body mass within *J. jacana*, due to aggressive interactions with a heterospecific competitor in sympatry. It is unlikely that this cline shift is due to ecological differences within and outside of the hybrid zone, as female *J. spinosa* body mass did not change across geography, whereas female *J. jacana* body mass significantly increased in sympatry.

A number of empirical studies suggest that asymmetry in competitive ability can facilitate differential introgression between lineages (reviewed in Lipshutz 2017a; Tinghitella et al. 2018). One case study example is a hybrid zone between subspecies of the common wall lizard (*Podarcis muralis*), in which asymmetries in male dominance lead to directional hybridization (MacGregor et al. 2017) and asymmetric introgression (While et al. 2015; Yang et al. 2018). An asymmetry in competitive ability can also facilitate hybrid zone movement via geographical displacement (Grava et al. 2012). Our findings broaden the framework of this literature on male competition and hybridization by presenting the first example of female competition facilitating introgression between hybridizing species. In the context of other hybrid zone studies, we find that intrasexual competition appears to have similar evolutionary outcomes whether the competitors are males or females, such that a female competitive trait is asymmetrically introgressed relative to genome-wide markers, from the direction of the more dominant into the less dominant species. Whether intrasexual competition is similar or different between the sexes has been debated recently (Tobias et al. 2012; Cain and Rosvall 2014). It has been argued that when females compete, they are more driven by access to high quality mates, whereas males are concerned more with quantity than quality of mates (Rosvall 2011). Given that competition is more important than mate choice in determining reproductive success in jacanas (Emlen and Wrege 2004a,b), we hypothesize that interspecific competition between females is more driven by access to mate quantity rather than quality.

For some hybrid zones in which intrasexual selection is the hypothesized mechanism driving introgression, the trait of interest has not been behaviorally linked to competitive interactions. For example, in a study hypothesizing that differential male aggression causes hybrid zone movement between hermit and Townsend's warblers, introgression of plumage color is interpreted as evidence of competition driving hybridization (Rohwer and Wood 1998; Pearson and Rohwer 2000). Although Townsend's warblers are more aggressive and have higher testosterone than hermit warblers, the plumage trait itself plays no known competitive function. In another instance, the

introgression of yellow plumage from golden-collared into white-collared manakins is explained by differential male aggression (McDonald et al. 2001) but also by female choice (Stein and Uy 2006). Thus, it is not clear whether inter- or intrasexual selection explains introgression in this system. In jacanas, female body mass is associated with female competition over breeding territories (and by extension, the males contained on those territories), but males do not appear to use female body mass in mate choice (Emlen and Wrege 2004b). Thus, the jacana hybrid zone represents one of the few studies in which there is evidence suggesting that competition, not mate choice, drives asymmetric introgression of a trait between hybridizing species. Long-term breeding observations of sympatric populations, along with paternity analyses, could test the hypothesis that larger bodied females monopolize harems of males in the hybrid zone. Future resampling of the hybrid zone should also assess whether the center has shifted, as recent work has found dramatic changes in hybrid zone structure across short time periods (Taylor et al. 2014; Ryan et al. 2018).

A recent debate in the sexual selection literature is whether traits that influence female–female competition, such as female body mass in jacanas, are sexually selected (Rosvall 2011). Here, we consider traits that influence competition for mates (either in terms of mate number or quality) as sexually selected, whereas traits that directly influence female fecundity or offspring survival are naturally selected. From a long-term study of *J. jacana* mating behavior at one site sampled on the transect, we know that females with larger body mass have larger territories, which allows them to include more mates in their harems and increase reproductive success (Emlen and Wrege 2004a; Emlen and Wrege 2004b). Sexual dimorphism is often a key indicator of sexual selection acting on a trait, and females in both species are 48–67% larger than their respective males (Emlen and Wrege 2004b; Lipshutz 2017b)– this dimorphism is more pronounced in *J. spinosa*. Female body mass is likely under sexual selection via female competition in these species, making it a strong candidate trait for asymmetric introgression due to interspecific competition for territories. In contrast, although jacanas use their wing spurs as weapons for fighting and in threat displays (Lipshutz 2017b), neither female nor male wing spur length significantly predicted reproductive success in the behavioral study (Emlen and Wrege 2004b). This could explain why these competitive traits were not as strongly asymmetrically introgressed relative to genome-wide markers in the hybrid zone. Another interpretation for dissimilar patterns of introgression is that these competitive traits play different roles in intra- versus interspecific competition. Differential introgression of sexual traits has been found in several other hybrid zones, for example song versus plumage in fairy wrens (Greig et al. 2015) and morphology/coloration versus pheromones in common wall lizards (MacGregor et al. 2017).

SELECTION AGAINST HYBRIDIZATION

We suspect intrinsic incompatibility selection against hybridization in the jacana hybrid zone. The presence of F1 hybrids and backcrosses suggests that gene flow is ongoing between the two species. The co-occurrence of both species near the current hybrid zone center was first described in 1924 by Griscom (Wetmore 1965), suggesting that secondary contact has been occurring for many generations (∼100 years or more). However, species boundaries are also being maintained, as we found few, mostly early generation backcrosses. Rarity of hybrids and predominance of parental forms suggests a bimodal hybrid zone (Jiggins and Mallet 2000). To infer selection against hybridization, the width of a cline should be interpreted relative to dispersal ability. A steep cline for genome-wide markers relative to high dispersal ability could suggest selection against hybridization between *J. spinosa* and *J. jacana* (e.g., tension zone). However, dispersal has not been well characterized for the family Jacanidae, although jacanas disperse locally with seasonality (Jenni and Mace 1999). Selection against hybrids could be the result of low hybrid fitness due to intrinsic postzygotic barriers such as Dobzhansky-Muller incompatibilities (Barton and Hewitt 1985), and future work should examine postzygotic barriers in jacanas.

Prezygotic barriers, such as species recognition traits, could also maintain species boundaries between jacanas by promoting reproductive isolation (Hudson and Price 2014), though this would not restrict the rest of the genome from introgressing, given that backcrossing occurs. We found that species-specific phenotypic traits, including facial ornamentation and dorsal plumage, had steep clines that were concordant with genome-wide markers, whereas the widths of clines associated with competitive traits were much wider. Across the Neotropical *J. jacana* species complex, all subspecies have chestnut-brown dorsal plumage except the black Panamanian *J. jacana* subspecies. This plumage difference in sympatry with a heterospecific is suggestive of reproductive character displacement. Whereas clines for plumage traits involved in reproductive isolation are often more narrow than genome-wide markers (Bailey et al. 2015; Semenov et al. 2017), it is possible that near-complete reproductive isolation due to intrinsic incompatibilities between the two jacana species results in similar patterns of introgression of plumage traits as background genome-wide markers.

Hybridization can promote the exchange of beneficial alleles between species despite the maintenance of species barriers across the majority of their genomes via adaptive introgression (Grant and Grant 2010). Recent examples include insecticide resistance in mosquitoes (Clarkson et al. 2014), color pattern in *Heliconius* butterflies (Enciso-Romero et al. 2017), and opsin genes and nuptial coloration in Lake Victoria cichlids (Meier et al. 2017; Meier et al. 2018). In jacanas, the adaptive potential of hybridization via introgression of female body mass could potentially outweigh the

costs of hybridization between the jacana species, as a larger body mass in females is associated with higher reproductive success (Emlen and Wrege 2004a; Emlen and Wrege 2004b). Comparing the introgression of putative loci for larger body mass, which is likely under positive selection, with loci resistant to introgression and associated with reduced hybrid viability, which indicate natural selection against hybridization, could lend insight into the potential roles of sexual and intrinsic incompatibility selection in the jacana hybrid zone.

CONCLUSIONS

In summary, we find the first evidence for differential introgression of a trait important in female–female competition relative to genome-wide markers. This trait is associated with reproductive success due to competition for territories and mates, not used in male mate choice, whereas male–male competition and female mate choice have been inferred to explain asymmetric introgression in other hybrid zone studies. We therefore suggest that competition between sex-role reversed females can facilitate hybridization, similar to males in systems with more typical sex roles.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Phenotypic examples of *Jacana jacana* (top), hybrids (middle), and *J. spinosa* (bottom) featuring (A) facial shield (B) wattles (C) wing spur and (D) dorsal plumage.

Figure S2. Genetic PC1 and Q (admixture proportions for STRUCTURE K = 2) are significantly and strongly positively correlated (r = 0.99, $p < 2.2 \times 10^{-16}$).

Figure S3. A pattern of isolation by distance was found in J. jacana (red) but not J. spinosa (yellow).

Figure S4. Individual variation in female body mass across the hybrid zone. Dotted vertical lines indicate cline centers for Q (STRUCTURE admixture proportion for K2) and female body mass.

Figure S5. Density plot cline centers for 8479 diagnostic, genome-wide loci in Hardy-Weinberg equilibrium (grey) relative to Q cline center (black) and female body mass cline center (purple).