

Divergent competitive phenotypes between females of two sex-role-reversed species

Sara E. Lipshutz¹ 

Received: 4 January 2017 / Revised: 6 June 2017 / Accepted: 12 June 2017
© Springer-Verlag GmbH Germany 2017

Abstract

Divergent phenotypes between lineages in the early stages of speciation can promote or impede reproductive isolation. Although divergence in male competitive morphology and behavior has been explored for many hybridizing lineages, it is less known how divergence between females influences hybridization. Here, I compare competitive phenotypes between females of two hybridizing, sex-role-reversed jacana species in Panama. Previous work suggests *Jacana spinosa* females monopolize mating in the hybrid zone, potentially through a competitive advantage. I tested whether *J. spinosa* females have a more competitive phenotype than *Jacana jacana* females. I compared morphological traits related to territoriality and measured aggressive behavior using territorial intrusion simulations: the first aggression assay in a shorebird. I also quantified these traits in males, to confirm previous studies reporting males as smaller and less aggressive than females in both species. As predicted, *J. spinosa* females had larger body mass and longer wing spurs than *J. jacana* females. *J. spinosa* females were also more aggressive than *J. jacana* females. Male *J. spinosa* had longer wing spurs than male *J. jacana*, but there was no difference in male body mass between the species, and *J. spinosa* males were more aggressive than *J. jacana* males. Additionally, male *J. spinosa* was

more aggressive than female *J. spinosa*, suggesting mixed support for females as competitively dominant to males and indicating the need for additional experimental work on sex differences in *Jacana*.

Significance statement

Historically, research on the role of mating behavior in hybridization has focused on competitive males and choosy females. Although female-female competition is widespread and has important fitness consequences for many species across numerous taxa, little is known about the role of female competition in mediating hybridization between closely related lineages. I compare morphological and behavioral traits related to competition between two hybridizing species of jacanas, tropical shorebirds with sex-role reversal. I find that *J. spinosa* females have a more competitive morphological phenotype and higher aggression than *J. jacana* females, which may allow them a competitive advantage in obtaining and defending territories and mates in the hybrid zone. These patterns align with a pattern of asymmetrical introgression of *J. spinosa* mitochondrial DNA previously described in the hybrid zone, as well as findings from other hybrid zones in which male-male competition can potentially explain asymmetric patterns of gene flow from the more dominant species into the less dominant species.

Communicated by S. Pruett-Jones

Electronic supplementary material The online version of this article (doi:10.1007/s00265-017-2334-0) contains supplementary material, which is available to authorized users.

✉ Sara E. Lipshutz
sara.lipshutz@gmail.com

¹ Department of Ecology and Evolutionary Biology, Tulane University, 400 Lindy Boggs, New Orleans, LA 70118, USA

Keywords Aggression · Female competition · Territorial intrusion · Sex-role reversal · Jacana · Shorebird

Introduction

For closely related lineages with similar life histories, divergent phenotypes may either promote or hinder reproductive isolation (Safran et al. 2013). Recently diverged species

provide good opportunities to investigate the function of divergent phenotypes in reproductive isolation, especially in the context of hybridization. The impact of divergent and sexually selected traits and preferences on reproductive isolation has been studied extensively in the context of mate choice between heterospecifics (Sætre et al. 1997; Wirtz 1999; Stein and Uy 2006; Baldassarre and Webster 2013) and is considered a signature of speciation by sexual selection (Safran et al. 2013). In addition to mate choice, divergent phenotypes are also important in mediating interspecific competition over territories, mates, and resources (Andersson 1994; Irwin and Price 1999; Grether et al. 2013). For example, differential aggression between hybridizing lineages can lead to displacement of the less aggressive species from breeding territories (Pearson and Rohwer 2000; Jankowski et al. 2010). This competitive exclusion can reduce gene flow, particularly when compounded with reproductive interference (Kishi et al. 2009; Drury and Grether 2014; but see Vallin et al. 2012). Alternatively, differential aggression can facilitate hybridization, for example, by driving genetic and/or phenotypic introgression into the less aggressive lineage (e.g., McDonald et al. 2001; Rosenfield and Kodric-Brown 2003; Grava et al. 2012; Robbins et al. 2014; While et al. 2015). Differential aggression between hybridizing species has been typically examined in males, leaving open the question of whether differential aggression in females may also affect hybridization.

Recent empirical and theoretical studies suggest that female-female competition is more widespread than previously thought (Rosvall 2011; Stockley and Bro-Jørgensen 2011; Tobias et al. 2012; Stockley and Campbell 2013). Intraspecific resource and mate defense by females has been demonstrated in numerous taxa (e.g., antelopes, Roberts and Dunbar 2000; birds, Rosvall 2008; lizards, While et al. 2009; and frogs, Meuche et al. 2011). However, a strong understanding of the ecological and evolutionary mechanisms that drive variation in female-female competition across closely related species is still lacking (Cain and Rosvall 2014). Excellent systems in which to examine this question are species for which traditional sex-roles are reversed. Differences in female competitive traits have known fitness consequences in sex-role-reversed species (Andersson 1995; Butchart 2000; Goymann et al. 2008), in contrast to species with traditional sex-roles, in which female competition is less well-studied. In comparison to males, females in role-reversed species are larger, often more brightly colored, and show higher levels of resource defense aggression (Cockburn 2006)—attributes typical of males in other species. In understanding the role that interspecific female competition plays in promoting or impeding speciation, a first step is to understand how female competitive traits vary among closely related species.

Several species of tropical shorebirds in the family Jacanidae exhibit classic examples of sex-role reversal, including female-biased size dimorphism (Jenni and Collier

1972; Butchart et al. 1999; Emlen and Wrege 2004a). Wattled jacana (*Jacana jacana*) females have a more competitive morphological phenotype than males—they are heavier and show greater proportionate development of weaponry relative to body size (Emlen and Wrege 2004b). In *J. jacana* and a closely related species, the Northern jacana (*Jacana spinosa*), both sexes have keratinized spurs used as weapons in aggressive interactions (Osborne and Bourne 1977; Emlen and Wrege 2004b). Body mass and tarsus length are strong predictors of female mating success in *J. jacana*, and only the largest females control access to mates by defending territories (Emlen and Wrege 2004a, b). Aggressive behavior in female jacanas has been observed in the context of acquisition and maintenance of male territories—males compete over territorial boundaries first, and female territories then encompass male territories (Emlen et al. 1989). Although some observational studies suggest that female jacanas are more aggressive than males because of their competitive dominance over territories (Jenni and Collier 1972; Stephens 1984), this has not been tested experimentally.

As female competition has consequences for reproductive success within jacana species, variation in competitive traits between females of sympatric jacana species may have implications for reproductive isolation between them. Two closely related jacana species, the Northern jacana and the wattled jacana, are known to hybridize in Panama (Miller et al. 2014). There is some evidence of asymmetrical introgression—hybrids shared mitochondrial DNA (mtDNA) haplotypes with *J. spinosa*, but not *J. jacana* (Miller et al. 2014). Because mtDNA is inherited maternally, one hypothesis to explain this asymmetrical introgression is that *J. spinosa* females monopolize mating in the hybrid zone (Miller et al. 2014). A behavioral mechanism by which *J. spinosa* females could monopolize successful matings is by having a more competitive phenotype that allows them to outcompete *J. jacana* females for territories. Here, I define the competitive phenotype as the covariance of competitive traits, in this case morphological and behavioral traits, involved in competition over mates and territories (West-Eberhard 1983; Andersson 1994; Cain and Ketterson 2012). Although a direct comparison of competitive morphology has not been made between the two species, sexual dimorphism is greater in *J. spinosa* (female to male mass ratio 1.67:1, Jenni and Collier 1972), than in *J. jacana* (mass ratio 1.48:1, Emlen and Wrege 2004a). It is unknown whether females of the two species are also different in behavioral characteristics of the competitive phenotype, for instance, their aggression levels.

In this study, I test the hypothesis that competitive morphological traits and aggressive behaviors are divergent between *J. spinosa* and *J. jacana* females and males. I predict that *J. spinosa* females have more competitive morphological traits than *J. jacana* females and will be more aggressive to territorial intruders. I also describe the first aggression assay using

simulated territorial intrusion in shorebirds. I quantify the same morphological traits and aggressive behaviors in females and males of both species, to place the female competitive phenotype in context and to examine a long-standing assumption that female jacanas are generally more aggressive than males in territory defense (e.g., Stephens 1984; Betts and Jenni 1991). Comparing competitive morphological traits and aggressive behaviors between the two species and sexes expands our knowledge of variation in the *Jacana* competitive phenotype and provides a first step towards understanding the potential role of female competition in hybridization.

Methods

Morphological measurements

Birds were captured with mist nets from April to May in 2012, June to September 2014, and May to August 2015, from Costa Rica to Panama (Fig. 1; Online Resource 1). I measured morphological traits from 165 individual adult jacanas (80 *J. jacana* and 85 *J. spinosa*). Individuals were aged based on plumage (Jenni 1996). I measured left and right keratinous wing spurs and tarsi to the nearest tenth of a millimeter with Avinet dial plastic calipers and then averaged for each individual. To obtain a combined metric of the competitive morphological phenotype for jacanas, I summarized body mass, tarsus length, and spur length using a principal component analysis (PCA) in R 3.2.2 (R Core Team 2015). Prior to the PCA, I log transformed behavioral responses to fulfill assumptions of multi-normality. I retained one PC score with an eigenvalue greater than 1 (hereafter morphological PC1), which explained 79.1% of the variation in competitive morphology (Table 1). I also calculated female to male mass ratios to compare measurements with other published studies (Jenni and Collier 1972; Emlen and Wrege 2004a).

Sex determination

Individuals were sexed based on mass (Wrege and Emlen 2005) and the presence of brood patches underneath the wings, in the case of males. I measured body mass to the nearest tenth of a gram with a Pesola® spring scale. While Wrege and Emlen (2005) identify a 100–108-g range where male and female masses may overlap, there exists a female *J. jacana* museum specimen with a body mass of 106.7 g (LSU 164012) and a male *J. spinosa* specimen in my dataset with a mass of 117 g (SL 188), suggesting that the range of mass overlap may be greater between the sexes. To confirm the sex of 46 jacanas with body masses ranging from 100 to 130 g and no brood patches, I used molecular techniques. I collected blood samples with brachial venipuncture and stored them in Queen's lysis buffer (Seutin et al. 1991). I extracted genomic

DNA with a DNeasy extraction kit (Qiagen) following the manufacturer's protocol. I amplified the CHD1Z gene using the primers 2550F/2718R (Fridolfsson and Ellegren 1999) in a polymerase chain reaction (PCR). For each 10- μ L reaction, I used 5 μ L of Qiagen Multiplex PCR Master Mix containing 3 mM MgCl₂ (Valencia, CA), 2 μ L of molecular-grade water, and 1 μ L of a 2 μ M primer mix. I used the following cycling parameters: 1 cycle of 15 min at 95 °C; 35 cycles of 30 s at 94 °C, 90 s at 52 °C, and 60 s at 72 °C, followed by 1 cycle of 30 min at 60 °C; and 1 cycle of 1 min at 25 °C. I ran the PCR products for 60 min on a 2% agarose gel stained with SYBR™ Safe (Invitrogen) and assigned sex based on differences in banding patterns between males and females. There were 16 males sampled from across both species with a body mass higher than 108 g, including two males at 124 g, which I would have misidentified if sexed based on mass alone.

Aggression assay experimental design

I measured aggression experimentally by simulating territorial intrusion with a taxidermic mount and auditory stimulus. Similar assays of aggression have been conducted on both males and females in a number of avian taxa, primarily in songbirds (Aves: Passeriformes) (Pearson and Rohwer 2000; Uy et al. 2009; Greig et al. 2015). To assay aggression in jacanas, I modified these standard methods using a moving visual stimulus to help the territory holder locate the simulated intruder (Online Resource 2).

Four female taxidermic mounts were prepared per species (eight in total), collected outside of the hybrid zone. Mounts were positioned in an identical aggressive stance, with their wings raised upwards, spurs exposed, and necks forward. To facilitate movement, I fixed mounts to a rotating wheel with strings and pulled them from 20 m away behind a blind, where I and another observer conducted behavioral observations. Because the natural coloration of the facial shield and wattles fades when dried, I painted the fleshy bare red ornaments of *J. jacana* with “Deep Red” paint and the bare yellow facial ornament of *J. spinosa* with “Brilliant Yellow” paint from a Crayola six-color acrylic paint set (manufacturer number 201997). There are no known visual signals in jacana facial ornaments that are not visible to the human eye, such as UV signaling. No changes were made to the green wing primary feathers of either species, which contain turacoverdin pigment (Bleiweiss 2015).

I used two types of auditory stimuli in the aggression assay—a lure to attract a territorial pair to the mount and a vocal stimulus played during the assay. The lure was a 10-s recording of a pair raucously calling in unison (e.g., Amy et al. 2010; Anderson et al. 2013). Vocal stimuli consisted of repeated-note calls (Mace 1981) of single females responding to conspecific playback. Vocalizations were recorded using a Sennheiser ME67 shotgun microphone and a

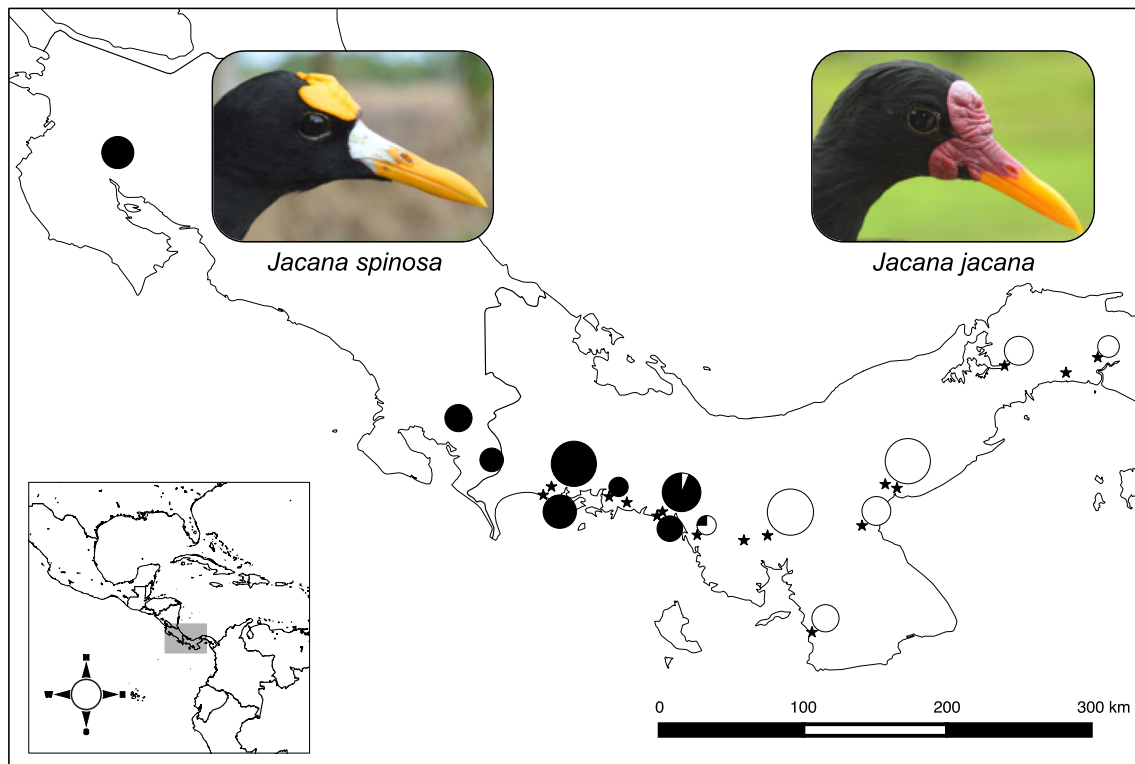


Fig. 1 Sampling map of Costa Rica and Panama. Circle size refers to number of individuals with morphological measurements, ranging from four to 24 individuals, and fill represents *J. spinosa* (black) and *J. jacana* (white). Stars represent sites that were locations of aggression assays

Marantz PMD661 MKII compact flash recorder (Saul Mineroff Electronics). Recordings were made at a 44.1-kHz sampling rate with 16-bit precision as .wav files. I also used two recordings of *J. spinosa* calls from Costa Rica (XC140613, XC72325) and one of *J. jacana* from Peru (XC47715) downloaded from Xeno Canto (www.xeno-canto.com), for which sex was unknown. I chose 5 s of high-quality calls from each recording, a typical duration for a repeated-note call bout (Jenni et al. 1974; Mace 1981; SEL unpublished data). The natural rate of call bouts for both species ranges from two to nine bouts/min (SEL unpublished data). Background noise was minimized using a band filter in Raven Pro 1.4 (Bioacoustics Research Program 2011) and noise reduction in Audacity® 2.1.0. Call bouts were alternated with 5 s of silence to create 9-min tracks of six call bouts/min,

with four different tracks per species (eight in total). Vocal tracks were played at 83–85 dB SPL 1 m from a Bluetooth speaker (Bose Mini SoundLink). I used both conspecific lures and vocal stimuli for each species, and all vocalizations were recorded outside of the hybrid zone.

Aggression assay protocol

From June to August 2015, I conducted aggression assays on 33 mated pairs of *J. jacana* and 28 mated pairs of *J. spinosa* (Fig. 1). These assayed individuals were different from those for which I took morphological measurements—thus, I do not have a direct comparison of aggressive responses and morphological measurements for each individual. I observed focal pairs over a 2-day period prior to the trial, to determine

Table 1 Mean and standard error for morphological traits related to aggression of adult male and female *Jacana spinosa* and *J. jacana*

Trait	PC1	<i>Jacana spinosa</i>		<i>Jacana jacana</i>	
		F	M	F	M
Eigenvalue	1.78				
Percent variation	79.2				
Body mass (g)	0.61	172.7 ± 2.7	103.2 ± 1.0	159.0 ± 2.7	105.9 ± 1.2
Average spur (mm)	0.56	14.1 ± 0.5	9.6 ± 0.2	11.2 ± 0.4	8.3 ± 0.2
Tarsus length (mm)	0.56	61.8 ± 0.4	56.3 ± 0.2	60.9 ± 0.5	55.3 ± 0.4
Sample size		35	50	28	52

territorial status, pair status, reproductive status, and territory boundaries (Emlen and Wrege 2004a). Because I identified distinct pairs by their territory locations, I avoided testing adjacent territories for which I could not distinguish the territory holders. This ensured that I did not assay the same individuals twice. In the case of a female with multiple male mates, I tested only the male whose territory was occupied by the female at the time of the assay. I also avoided testing pairs for which a male was incubating a nest or there were chicks present, as that could influence aggression levels. I tested pairs using a randomized combination of four conspecific taxidermic female mounts and four conspecific vocalizations per species. Mount and stimulus combinations were presented in a randomized order across pairs.

Prior to beginning each trial, I placed a mount and speaker in the center of each territory and green flags at distances of 2 and 8 m from the mount. I positioned the mount on a 15-cm-high platform and elevated the speaker at 1 m to project above tall vegetation. At the start of an assay, I played the lure until both focal individuals approached within 20 m of the mount. All pairs responded within eight plays of the lure. Once both individuals were within 20 m of the mount, I waited 1 min and then started the trial with the 9-min recording of conspecific vocalizations. For the first 30 s of vocalizations, I rotated the mount (Online Resource 2).

I and an assistant observed each focal individual and recorded behavioral observations using handheld recorders. It was not possible to record data blind because this study involved focal animals in the field. Observers continuously recorded aggressive behaviors towards the mount, including pecks, flyovers, wing raises, threats, and hoverflights according to ethograms developed for *J. jacana* (Altmann 1974; Emlen and Wrege 2004b) and *J. spinosa* (Jenni and Betts 1978; Stephens 1984) (all responses listed in Table 2). We split each trial into 10-s intervals based on the start of the repeated vocal stimulus. For each 10-s interval, we scored a focal individual as vocalizing if it called any number of times within the interval. We also measured distance to the mount at each 10-s interval in three distance categories: 0–2, 2–8, or 8–20 m. To calculate average distance to the mount, I counted the number of instances an individual was in each distance category for all 10-s intervals, multiplied this count by the midpoint of these distance categories, and divided by the number of 10-s intervals for the entire trial. We also recorded latency to approach within 0–2 m, latency to approach within 2–8 m, and the proportion of time spent within 0–2 m. I excluded from analyses the 10-s intervals for which an individual was not observed, including when it was greater than 20 m from the mount, because visibility was sometimes reduced in tall grass. For this reason, continuous behaviors and vocalizations are presented as proportions of the total 10-s intervals for which individual location was known. I calculated this as frequency divided by the number of 10-s intervals, and the rate of

vocalization as the number of 10-s intervals for which an individual was calling.

To compare aggression between the species, I summarized the behavioral responses using a PCA in R; I log transformed behavioral responses to fulfill assumptions of multi-normality. I retained three PC scores (eigenvalues >1) that explained 61.4% of the variation among territory holders in their response to a simulated intruder (Table 2). I used each PC score (hereafter “aggressions PC1, PC2, and PC3”) as a dependent variable in subsequent comparisons of sex and species.

Model formation and selection

I ran linear mixed effects models using the *lme4* package (Bates et al. 2015) in R. I visually inspected residual plots to ensure that they did not deviate from normality and used the `varIdent` function to account for heteroscedasticity in both species and sexes to meet model assumptions. I compared models using a type-III ANOVA and AIC_c to account for low sample sizes using the *AICcmodavg* package in R (Mazerolle 2016) (Online Resources 3 and 4). I used a type-III ANOVA to determine which fixed effects were significant predictors of response variables and a Tukey’s post hoc test to compare within and between the sexes and species using the *multcomp* package in R (Hothorn et al. 2008).

To compare morphological traits used in competitive interactions between both species and sexes, I used the morphological PC1 as the response variable; specified species, sex, and the interaction between species and sexes as fixed effects; and included site and year as random effects.

To compare aggression between both species and sexes, I used aggression PC1, PC2, and PC3 as separate response variables and species, sex, and the interaction between species and sexes as fixed effects. To account for mount/vocal stimulus exemplar effects, I included mount ID and vocal stimulus as random effects nested within species. I also included pair as a random effect nested within site, because within a pair, male and female distances from the mount were positively correlated (Pearson correlation $r = 0.655$).

Results

Competitive morphology varies with species and sex

Morphological PC1 was positively associated with body mass (0.61), tarsus length (0.56), and spur length (0.56) (Table 1). The best-supported model for morphological PC1 included all fixed effects and site as the sole random effect (Online Resource 3). Species ($F_{1,145} = 14.6$, $P = 0.0002$), sex ($F_{1,145} = 207.16$, $P < 0.0001$), and their interaction ($F_{1,145} = 6.93$, $P = 0.0094$) were all significant predictors of

Table 2 Mean \pm SE behavioral responses to a conspecific and simulated territorial intruder and their principal component score loadings

Sex	PC1	PC2	PC3	<i>Jacana spinosa</i>		<i>Jacana jacana</i>	
				F	M	F	M
Eigenvalue	1.84	1.21	1.14				
Percent variation	33.8	14.6	13				
Cumulative proportion	33.8	48.4	61.4				
Latency 0–2 m (s)	-0.37	0.21	-0.05	422 \pm 35.43	343.6 \pm 41.13	502.86 \pm 22.31	474.33 \pm 31.27
Latency 2–8 m (s)	-0.4	0.02	-0.17	210.4 \pm 41.64	190.4 \pm 41.43	417.86 \pm 38.83	375 \pm 43.58
Proportion time 0–2 m	0.44	-0.28	0.18	0.10 \pm 0.038	0.19 \pm 0.058	0.0053 \pm 0.0033	0.014 \pm 0.008
Mean distance	-0.46	0.17	-0.24	10.81 \pm 0.64	9.15 \pm 0.89	13.10 \pm 0.39	12.95 \pm 0.38
Proportion hoverflights	0.26	0.49	-0.14	0.014 \pm 0.01	0.036 \pm 0.01	0.013 \pm 0.003	0.038 \pm 0.01
Proportion wing spreads	0.29	0.39	-0.08	0.031 \pm 0.01	0.13 \pm 0.03	0.018 \pm 0.004	0.020 \pm 0.01
Proportion threats	0.05	-0.13	-0.75	0	0.010 \pm 0.01	0.0007 \pm 0.001	0.0006 \pm 0.001
Proportion pecks	0.18	-0.46	-0.45	0	0.026 \pm 0.02	0.0027 \pm 0.002	0.0019 \pm 0.002
Proportion flyovers	0.21	0.04	-0.24	0.026 \pm 0.01	0.035 \pm 0.08	0.014 \pm 0.004	0.0093 \pm 0.003
Proportion vocalizations	0.27	0.48	-0.19	0.21 \pm 0.04	0.40 \pm 0.06	0.17 \pm 0.04	0.25 \pm 0.05
Sample size				25	25	28	30

Factors that load highly onto each PC are in bold

morphological PC1. Female *J. spinosa* has larger values of morphological PC1 than female *J. jacana* ($z = 3.82$, $P < 0.001$), indicating overall larger competitive traits (Table 1, Fig. 2). Comparison of individual morphological traits (Table 1) indicates that *J. spinosa* females have a larger body mass, longer keratinous wing spurs, and longer tarsi than *J. jacana* females. In contrast, males of both species did not have significantly different values of morphological PC1 ($z = 1.96$, $P = 0.194$). *J. spinosa* males have longer average wing spurs, however ($t = -5.01$, $P < 0.001$).

For both species, females had significantly larger values of morphological PC1 than males (*J. spinosa*, $z = 17.1$, $P < 0.001$; *J. jacana*, $z = -14.4$, $P < 0.001$). The differences in body mass were more extreme in *J. spinosa*, which had a female to male mass ratio of 1.67, than in *J. jacana*, which had a ratio of 1.5.

J. spinosa is more aggressive than *J. jacana*

The aggression score based on PC1, which explained 33.8% of variation in aggressive response, included latency to approach the mount, distance to the mount, and time spent close to the mount as important loading variables (Table 2). After model selection, the best-supported model for aggression PC1 included pair nested within site, as the sole random effect, and all fixed effects. Species ($F_{1,16} = 9.86$, $P = 0.0063$) and the interaction between species and sexes ($F_{1,46} = 8.16$, $P = 0.0064$) were significant predictors of aggression PC1, but sex alone was not significant ($F_{1,46} = 2.32$, $P = 0.134$). *J. spinosa* had higher aggression PC1 score than *J. jacana* for both females and males (female, $z = 3.14$, $P = 0.008$; male, $z = 4.33$, $P < 0.001$) (Fig. 3). Male *J. spinosa* had significantly

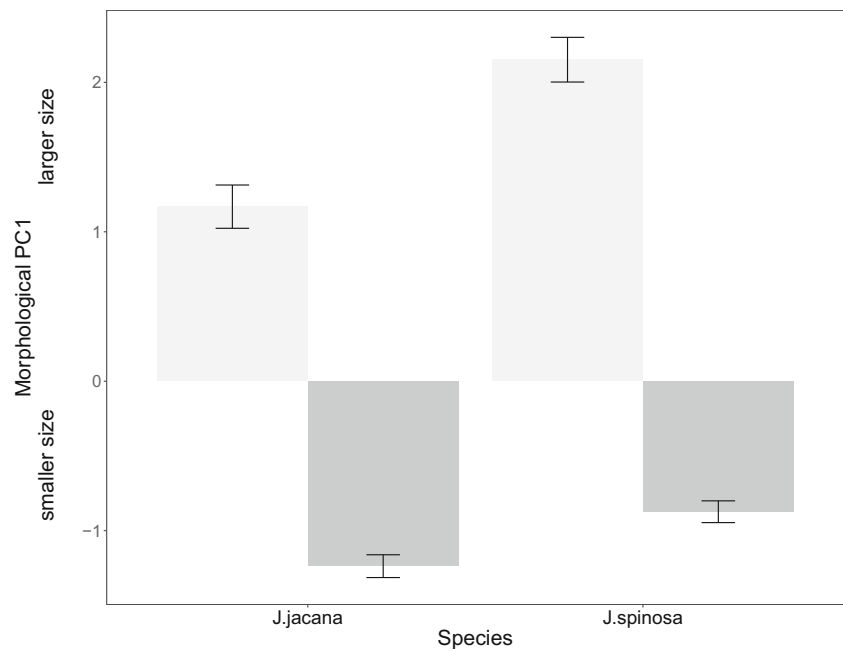
higher aggression PC1 scores than female *J. spinosa* ($z = 4.67$, $P < 0.001$), but there were no differences between male and female *J. jacana* aggression PC1 scores ($z = 1.52$, $P = 0.391$). No fixed effects were significant for either PC2 or PC3 (all $P > 0.09$) (Online Resource 4), which only explained a small proportion of the variation in aggressive response (Table 2).

Discussion

I found support for my prediction that *J. spinosa* females have a more competitive phenotype than *J. jacana* females. *J. spinosa* females on average had a larger body mass and longer wing spurs than *J. spinosa* females, traits involved in competition over territories and mates (Emlen and Wrege 2004b). Consistent with these differences in competitive morphological traits, I found that *J. spinosa* females behaved more aggressively towards a conspecific intruder in measures of distance and time spent near the mount than did *J. jacana* females. Male *J. spinosa* were also more aggressive than male *J. jacana* and had significantly longer wing spurs. Together, these morphological and behavioral data suggest that *J. spinosa* and *J. jacana* have diverged in their competitive phenotype. Additionally, I found mixed support for a long-standing hypothesis that females have a more competitive phenotype than males in the sex-role-reversed *Jacana* genus. As predicted, females were larger than males in both species; however, females were not more aggressive than males in either species. Below, I discuss the implications of these findings for our understanding of these sex-role-reversed species.

Differences in the female competitive phenotype between the species may be related to greater sexual selection on

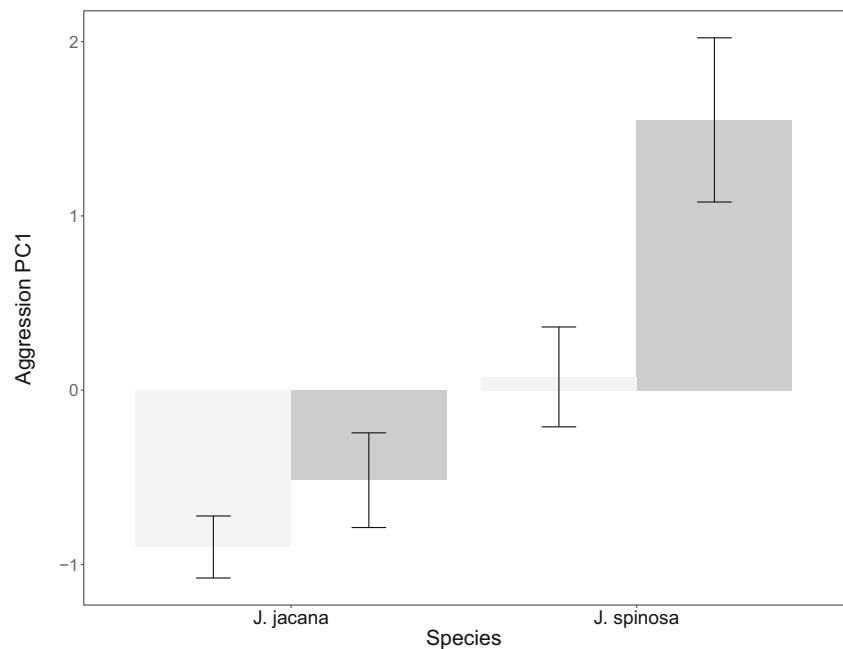
Fig. 2 Mean (\pm SE) morphological PC1 for female (*light gray*) and male (*dark gray*) *J. spinosa* and *J. jacana*



secondary sexual traits for *J. spinosa* females. Some evidence suggests stronger competition for mates and territories in *J. spinosa* than in *J. jacana*. For instance, sexual dimorphism in morphological traits is more pronounced in *J. spinosa* than in *J. jacana*, as demonstrated by this and other studies (Jenni and Collier 1972; Emlen and Wrege 2004b). Furthermore, within-population estimates suggest that breeding *J. spinosa* females have an average of 1.8–2.5 male mates in their territories (Jenni and Collier 1972; Jenni and Betts 1978), whereas *J. jacana* breeding females have an average of 1.6–1.7 mates (Emlen et al. 1998; Emlen and Wrege 2004b). Given that *J.*

spinosa has a higher average of male mates in a harem, and therefore higher potential for reproductive skew (Shuster 2009; Kvarnemo and Simmons 2013), the intensity of sexual selection may be stronger in this species. Therefore, I suggest that differences in the female competitive phenotype between *J. spinosa* and *J. jacana* are potentially the result of differences in sexual selection pressures. Future work to determine the ultimate and proximate mechanisms for these differences in competitive phenotype between closely related species could provide valuable insight into how and why young taxa diverge.

Fig. 3 Response by territory holders to simulated territorial intrusion of a conspecific. Mean (\pm SE) aggression scores (principal component 1) for *J. spinosa* (females $N = 25$; males $N = 25$) and *J. jacana* (females $N = 28$; males $N = 30$). *J. spinosa* males (*dark gray*) and females (*light gray*) responded more strongly than *J. jacana* males and females



This study represents the first experimental measure of aggression in jacanas. Contrary to previous studies, female jacanas were not more aggressive than male jacanas. *J. spinosa* males responded more aggressively than *J. spinosa* females towards a female conspecific intruder, but there were no differences in aggression between male and female *J. jacana*. Observational studies of aggression in *J. spinosa* (Stephens 1984) and in *J. jacana* (Emlen and Wrege 2004a, b) have suggested that female jacanas are dominant to males in territorial interactions because of their larger body size and therefore stronger resource-holding potential. In both species, female territories are larger and may encompass multiple male territories (Jenni and Collier 1972; Emlen et al. 1998). In a related species, the bronze-winged jacana (*Metopidius indicus*) males are less successful at evicting female intruders (Butchart et al. 1999). However, my results are somewhat consistent with the finding from observational studies that male jacanas are more responsive towards territorial intruders than females. For example, *J. jacana* males are more likely than females to respond first to both male and female conspecific intruders (Emlen and Wrege 2004a), and male *J. spinosa* are more responsive overall to non-jacana heterospecifics than are females (Stephens 1984). Male bronze-winged jacanas were more active than females in multiple measures of territory defense, including time spent alert, frequency of territory patrol, and hoverflights (Butchart et al. 1999). Although female jacanas in both species may be physically larger and dominant to males in resource-holding potential, males may be more responsive in aggressive territorial interactions.

Differences in morphology and aggression could allow *J. spinosa* females and males a competitive advantage over *J. jacana* in obtaining and defending territories where they come into contact in the hybrid zone. These morphological and behavioral results can be used to make predictions about the potential introgression of competitive traits and aggression across the hybrid zone. Evidence from other hybrid zones in species with male-dominant mating systems suggests that male aggression drives genetic introgression into the less aggressive species (McDonald et al. 2001; Grava et al. 2012; While et al. 2015). However, these studies did not specifically assay female aggression, and while there may be many systems in which differential female aggression acts in parallel or in opposition to differential male aggression, this has not been empirically investigated. Given that females in a diverse range of mating systems do compete for resources (see Rosvall 2011), this is an important avenue of future research. The current study indicates that both sexes can demonstrate differential aggression in secondary contact. Future work should investigate whether this drives introgression or reduces gene flow in the jacana hybrid zone. As jacanas are role-reversed and polyandrous, we might expect that higher aggression in both sexes of *J. spinosa* has different outcomes for hybrid zone dynamics. For example, *J. spinosa* females could

outcompete *J. jacana* females for territories encompassing potential mates irrespective of whether they are conspecific or heterospecific males, which could promote hybridization. In contrast, competition between males of the two species, especially the exclusion of *J. spinosa* by *J. jacana*, could reduce gene flow.

Interspecific divergence in the female competitive phenotype is one behavioral hypothesis that could explain the asymmetrical pattern of *J. spinosa* mtDNA introgression in the hybrid zone. However, divergence in male aggression would not explain this pattern. Future work should examine evidence for Haldane's rule, another hypothesis regarding asymmetric introgression of mtDNA whereby viability and/or fertility is reduced in the heterogametic sex, which in birds are females (e.g., Carling and Brumfield 2008). Additional work on the *Jacana* hybrid zone should also investigate character displacement in competitive morphology and aggressive behavior, as well as discriminatory behavior between the species. Increased divergence in sympatry could promote reproductive isolation and/or reduce competition between the species (Pfennig and Pfennig 2009). Character displacement could also be asymmetric, in which the species differ in the extent of their divergence in sympatry versus allopatry (Cooley 2007; Dingle et al. 2010; Pfennig and Stewart 2011), which could facilitate asymmetric introgression. The findings presented here provide insight into variation in the competitive phenotype in both sexes and open up new avenues of inquiry about the role that male and female competitions play in reproductive isolation between closely related lineages.

Acknowledgements Thanks to S. Pruett-Jones, two anonymous reviewers, and members of the Derryberry Lab writing workshop for useful comments on earlier drafts of this manuscript. Thanks to Gina Zwicky and Hannah Wilson of Tulane University and Pablo Gutierrez of the University of Panama who assisted with aggression assays. Daniel Buitrago, Jorge Medina, Pedro Caballero, and Jorge Garzon of the Smithsonian Tropical Research Institute Bird Collection (STRIBC) prepared the taxidermic mounts. Fahamida Khanam and Maliha Tabassum of the City College of New York transcribed audio recordings of the aggression assays. STRIBC and the LSU Museum of Natural Science Ornithology Collection provided specimen references for jacana body mass. S. Lantz assisted with PCR troubleshooting.

Compliance with ethical standards

Funding This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under grant no. 1154145, as well as a National Geographic Young Explorer Grant, Animal Behavior Student Grant, and Alexander Wetmore Grant from the American Ornithologists' Union. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the funding organizations.

Conflict of interest The author declares that she has no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Fieldwork was done with the prior approval of the Autoridad Nacional del Ambiente in Panama (permit nos. SE/A-45-12, SE/A-46-14, SE/A-36-15) and the Ministerio del Ambiente y Energía (MINAE- CONAGEBio) in Costa Rica. Blood samples for sexing were imported with Federal Fish and Wildlife permit MB26646B-0 to SEL and USDA APHIS VS16-3 permit no.123392 to Dr. Maggie MacPherson, with her permission. All works were approved by the Institutional Animal Care and Use Committee of the Smithsonian Tropical Research Institute (IACUC permit 2012-0315-2015) and Tulane University (IACUC permit 0446R).

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

- Altmann J (1974) Observational study of behaviour: sampling methods. *Behaviour* 49:227–267
- Amy M, Sprau P, de Goede P, Naguib M (2010) Effects of personality on territory defence in communication networks: a playback experiment with radio-tagged great tits. *Proc R Soc Lond B* 277:3685–3692
- Anderson RC, DuBois AL, Piech DK, Searcy WA, Nowicki S (2013) Male response to an aggressive visual signal, the wing wave display, in swamp sparrows. *Behav Ecol Sociobiol* 67:593–600
- Andersson M (1994) Sexual selection. Princeton University Press, Princeton
- Andersson M (1995) Evolution of reversed sex roles, sexual size dimorphism, and mating system in coucals (Centropodidae, Aves). *Biol J Linn Soc* 54:173–181
- Baldassarre DT, Webster MS (2013) Experimental evidence that extra-pair mating drives asymmetrical introgression of a sexual trait. *Proc R Soc B* 280:20132175
- Bates D, Maechler M, Bolker BM, Walker S (2015) Fitting linear mixed-effects models using {lme4}. *J Stat Softw* 67:1–48
- Betts BJ, Jenni DA (1991) Time budgets and the adaptiveness of polyandry in the Northern Jacana. *Wilson Bull* 103:578–597
- Bioacoustics Research Program (2011) Raven Pro: interactive sound analysis software. www.birds.cornell.edu/raven
- Bleiweiss R (2015) Extrinsic versus intrinsic control of avian communication based on colorful plumage porphyrins. *Evol Biol* 42:483–501
- Butchart SHM (2000) Population structure and breeding system of the sex-role reversed, polyandrous Bronze-winged Jacana *Metopidius indicus*. *Ibis* 142:93–102
- Butchart SHM, Seddon N, Ekstrom JMM (1999) Polyandry and competition for territories in Bronze-winged Jacanas. *J Anim Ecol* 68:928–939
- Cain KE, Ketterson ED (2012) Competitive females are successful females; phenotype, mechanism and selection in a common songbird. *Behav Ecol Sociobiol* 66:241–252
- Cain KE, Rosvall KA (2014) Next steps for understanding the selective relevance of female-female competition. *Front Ecol Evol* 2:32
- Carling MD, Brumfield RT (2008) Haldane's rule in an avian system: using cline theory and divergence population genetics to test for differential introgression of mitochondrial, autosomal, and sex-linked loci across the *Passerina* bunting hybrid zone. *Evolution* 62:2600–2615
- Cockburn A (2006) Prevalence of different modes of parental care in birds. *Proc R Soc Lond B* 273:1375–1383
- Cooley JR (2007) Decoding asymmetries in reproductive character displacement. *P Acad Nat Sci Phila* 156:89–96
- Dingle C, Poelstra JW, Halfwerk W, Brinkhuizen DM, Slabbekoorn H (2010) Asymmetric response patterns to subspecies-specific song differences in allopatry and parapatry in the gray-breasted woodwren. *Evolution* 64:3537–3548
- Drury JP, Grether GF (2014) Interspecific aggression, not interspecific mating, drives character displacement in the wing coloration of male rubyspot damselflies (*Hetaerina*). *Proc R Soc B* 281:20141737
- Emlen ST, Wrege PH (2004a) Division of labour in parental care behaviour of a sex-role-reversed shorebird, the Wattled Jacana. *Anim Behav* 68:847–855
- Emlen ST, Wrege PH (2004b) Size dimorphism, intrasexual competition, and sexual selection in Wattled Jacana (*Jacana jacana*), a sex-role-reversed shorebird in Panama. *Auk* 121:391–403
- Emlen ST, Demong NJ, Emlen DJ (1989) Experimental induction of infanticide in female Wattled Jacanas. *Auk* 41:225–230
- Emlen ST, Wrege PH, Webster MS (1998) Cuckoldry as a cost of polyandry in the sex-role-reversed Wattled Jacana, *Jacana jacana*. *Proc Biol Sci* 265:2359
- Fridolfsson A-K, Ellegren H (1999) A simple and universal method for molecular sexing of non-ratite birds. *J Avian Biol* 30:116–121
- Goymann W, Wittenzellner A, Schwabl I, Makomba M (2008) Progesterone modulates aggression in sex-role reversed female African black coucals. *Proc R Soc Lond B* 275:1053–1060
- Grava A, Grava T, Didier R, Lait LA, Dosso J, Koran E, Burg TM, Otter KA (2012) Interspecific dominance relationships and hybridization between black-capped and mountain chickadees. *Behav Ecol* 23:566–572
- Greig EI, Baldassarre DT, Webster MS (2015) Differential rates of phenotypic introgression are associated with male behavioral responses to multiple signals. *Evolution* 69:2602–2612
- Grether GF, Anderson CN, Drury JP, Kirschel ANG, Losin N, Okamoto K, Peiman KS (2013) The evolutionary consequences of interspecific aggression. *Ann N Y Acad Sci* 1289:48–68
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363
- Irwin DE, Price T (1999) Sexual imprinting, learning and speciation. *Heredity* 82:347–354
- Jankowski JE, Robinson SK, Levey DJ (2010) Squeezed at the top: interspecific aggression may constrain elevational ranges in tropical birds. *Ecology* 91:1877–1884
- Jenni DA (1996) Family Jacanidae (Jacanas). In: del Hoyo J, Elliot A, Sargatal J (eds) Handbook of the birds of the world. Lynx Edicions, Barcelona, pp 276–287
- Jenni DA, Betts BJ (1978) Sex differences in nest construction, incubation, and parental behaviour in the polyandrous American Jacana (*Jacana spinosa*). *Anim Behav* 26:207–218
- Jenni DA, Collier G (1972) Polyandry in the American Jacana. *Auk* 89:743–765
- Jenni DA, Gamba RD, Betts BJ (1974) Acoustic behavior of the Northern Jacana. *Living Bird* 13:193–210
- Kishi S, Nishida T, Tsubaki Y (2009) Reproductive interference determines persistence and exclusion in species interactions. *J Anim Ecol* 78:1043–1049
- Kvarnemo C, Simmons LW (2013) Polyandry as a mediator of sexual selection before and after mating. *Philos T Roy Soc B* 368:20120042
- Mace TR (1981) Causation, function, and variation of the vocalizations of the Northern Jacana (*Jacana spinosa*). PhD thesis, University of Montana, Missoula

- Mazerolle MJ (2016) Model selection and multimodel inference based on (Q)AIC(c). R Package version 2.0-4, <https://cran.r-project.org/web/packages/AICcmodavg/AICcmodavg.pdf>
- Mcdonald DB, Clay RP, Brumfield RT, Braun MJ (2001) Sexual selection on plumage and behavior in an avian hybrid zone: experimental tests of male-male interactions. *Evolution* 55: 1443–1451
- Meuche I, Linsenmair KE, Pröhl H (2011) Female territoriality in the strawberry poison frog (*Oophaga pumilio*). *Copeia* 2011:351–356
- Miller MJ, Lipshutz SE, Smith NG, Bermingham E (2014) Genetic and phenotypic characterization of a hybrid zone between polyandrous Northern and Wattled Jacanas in Western Panama. *BMC Evol Biol* 14:227
- Osborne DR, Boume GR (1977) Breeding behavior and food habits of the Wattled Jacana. *Condor* 79:98–105
- Pearson SF, Rohwer S (2000) Asymmetries in male aggression across an avian hybrid zone. *Behav Ecol* 11:93–101
- Pfennig KS, Pfennig DW (2009) Character displacement: ecological and reproductive responses to a common evolutionary problem. *Q Rev Biol* 84:253–276
- Pfennig KS, Stewart AB (2011) Asymmetric reproductive character displacement in male aggregation behaviour. *Proc R Soc Lond B* 278: 2348–2354
- R Core Team (2015) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna <https://www.r-project.org/>
- Robbins TR, Walker LE, Gorospe KD, Karl SA, Schrey AW, McCoy ED, Mushinsky HR (2014) Rise and fall of a hybrid zone: implications for the roles of aggression, mate choice, and secondary succession. *J Hered* 105:226–236
- Roberts SC, Dunbar RIM (2000) Female territoriality and the function of scent-marking in a monogamous antelope (*Oreotragus oreotragus*). *Behav Ecol Sociobiol* 47:417–423
- Rosenfield JA, Kodric-Brown A (2003) Sexual selection promotes hybridization between Pecos pupfish, *Cyprinodon pecosensis* and sheepshead minnow, *C. variegatus*. *J Evol Biol* 16:595–606
- Rosvall KA (2008) Sexual selection on aggressiveness in females: evidence from an experimental test with tree swallows. *Anim Behav* 75:1603–1610
- Rosvall KA (2011) Intrasexual competition in females: evidence for sexual selection? *Behav Ecol* 22:1131–1140
- Sætre G, Moum T, Bures S, Král N, Adamjan M, Moreno J (1997) A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* 387:1995–1998
- Safran RJ, Scordato ESC, Symes LB, Rodríguez R, Mendelson TC (2013) Contributions of natural and sexual selection to the evolution of premating reproductive isolation: a research agenda. *Trends Ecol Evol* 28:643–650
- Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Can J Zool* 69:82–90
- Shuster SM (2009) Sexual selection and mating systems. *P Natl Acad Sci USA* 106:10009–10016
- Stein AC, Uy JAC (2006) Unidirectional introgression of a sexually selected trait across an avian hybrid zone: a role for female choice? *Evolution* 60:1476–1485
- Stephens ML (1984) Aggressive behavior of the polyandrous Northern Jacana (*Jacana spinosa*). *Auk* 101:508–518
- Stockley P, Bro-Jørgensen J (2011) Female competition and its evolutionary consequences in mammals. *Biol Rev* 86:341–366
- Stockley P, Campbell A (2013) Female competition and aggression: interdisciplinary perspectives. *Philos T Roy Soc B* 368:20130073
- Tobias JA, Montgomerie R, Lyon BE (2012) The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philos T Roy Soc B* 367:2274–2293
- Uy JAC, Moyle RG, Filardi CE, Cheviron ZA (2009) Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the melanocortin-1 receptor. *Am Nat* 174:244–254
- Vallin N, Rice AM, Bailey RI, Husby A, Qvarnström A (2012) Positive feedback between ecological and reproductive character displacement in a young avian hybrid zone. *Evolution* 66:1167–1179
- West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. *Q Rev Biol* 58:155–183
- While GM, Sinn DL, Wapstra E (2009) Female aggression predicts mode of paternity acquisition in a social lizard. *Proc R Soc Lond B* 276: 2021–2029
- While GM, Michaelides S, Heathcote RJP et al (2015) Sexual selection drives asymmetric introgression in wall lizards. *Ecol Lett* 18:1366–1375
- Wirtz P (1999) Mother species-father species: unidirectional hybridization in animals with female choice. *Anim Behav* 58:1–12
- Wrege PH, Emlen ST (2005) Sexing criteria, accuracy, and statistical inference—a reply. *Auk* 122:347–349