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Variation in flight morphology in a damselfly with female-limited polymorphism

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ABSTRACT

Background: Female-limited colour polymorphisms occur in many species of dragonflies and damselflies. Often one female morph appears male-like in coloration (androchromes) whereas one or more others are distinct from males (gynochromes). These androchromes are hypothesized to be male-mimics, thereby avoiding the harassment of excessive male mating attempts.

Organism: The damselfly *Ischnura ramburii*, Rambur's fork-tail, is a widespread New World species with androchrome and gynochrome females. It was introduced to the Hawaiian Islands in the mid-1970s and females were thought to be exclusively gynochromatic there.

Questions: How do males and females differ in their flight apparatus? Do females with different colour morphologies also differ in flight morphology?

Hypothesis: Because male-like coloration is sometimes associated with male-like flight behaviours, androchrome females should have more male-like wings than gynochrome females.

Methods: We caught individuals of *I. ramburii* in the field from seven populations on three of the Hawaiian Islands and three populations in Texas (part of its native range). Using digitized wing and body images, we compared body size, wing size, and wing shape between sexes, between female morphs, and among geographic regions.

Results: Male *I. ramburii* are smaller than females and have smaller, more slender wings. Although androchromes are absent from the Big Island of Hawaii, both androchrome and gynochrome females are common on Oahu and Kauai. Androchrome females are indistinguishable from gynochrome females in all aspects of their flight apparatus except for forewing size, which is smaller than that of gynochromes and thus more-male like. Wing shape and size vary geographically. Body- and wing-size differences between males and females are consistent across regions, although the degree and direction of sexual dimorphism in wing shape are not.

Keywords: female-limited polymorphism, flight morphology, *Ischnura ramburii*, male mimicry, Rambur's fork-tail.

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INTRODUCTION

When males and females experience different costs and benefits from mating, the scene is set for sexually antagonistic co-evolution. In females, a variety of strategies can evolve to reduce the costs of unsolicited male interaction, sometimes resulting in sexual dimorphism or sex-limited polymorphism. Female-limited polymorphism occurs in many species of damselflies, and is often attributed to sexual conflict (Svensson *et al.*, 2007). In over 100 species of Odonates, some females resemble males in coloration ('androchromes') whereas others ('gynochromes') do not (Fincke *et al.*, 2005). It has long been thought that androchromes might benefit from reduced harassment as a result of their resemblance to male conspecifics ['male mimicry' (Robertson, 1985)], at the potential cost of remaining unmated when males are rare. More recent work, however, shows that morphs can differ in traits that are apparently unrelated to mimicry (e.g. Takahashi and Watanabe, 2010), and that other selective pressures may also contribute to the maintenance of female-limited polymorphism (e.g. Cooper, 2010).

The potential for sexual conflict to drive diversification of damselfly morphology and behaviour is supported by key features of damselfly reproduction. After a single mating, females typically acquire and store sufficient sperm for the entire breeding season (e.g. Sirot and Brockmann, 2001). Although females may benefit from multiply mating if they exhibit cryptic post-copulatory female choice (as suggested by Eberhard, 1997; Fincke *et al.*, 1997; Córdoba-Aguilar, 1999), subsequent matings can impose costs such as reduced fecundity, increased predator exposure, and compromised offspring quality, thereby reducing female fitness (Cordero, 1992; Sirot and Brockmann, 2001). By contrast, male damselflies gain potential fitness returns from any mating, including pairings with previously inseminated females, due to strong last-male precedence (Waage, 1986). Thus, a conflict over re-mating may exist between the sexes, which may have important consequences for population dynamics and phenotypic evolution.

In the absence of selection, drift could be relied upon to eventually remove one or the other morph from the population (Van Gossum *et al.*, 2008). Robertson (1985) proposed that conflicts over mating rate could promote female polymorphism if androchrome females, by effectively mimicking males, avoid unsolicited copulation attempts ('male mimicry hypothesis'). This reduction in harassment may come at the cost of remaining unmated when males are rare, promoting polymorphisms between gynochromes and androchromes.

Other investigators have suggested instead that frequency-dependent harassment of gynochromes and androchromes maintains polymorphism ['frequency dependent hypothesis' or 'learned mate recognition' (Miller and Fincke, 1999)]. Male preferences for androchrome and gynochrome phenotypes depend upon prior experience with females (e.g. Miller and Fincke, 1999; Van Gossum, 2001; Fincke *et al.*, 2007), thus polymorphism could result from males simply preferring the prevailing morph within a population, independent of mimicry (e.g. Miller and Fincke, 1999). Similarly, under the male mimicry hypothesis, the effectiveness of male mimicry could decline when androchromes exceed a threshold frequency and males learn to recognize them as potential mates.

An important difference between hypotheses invoking male mimicry (e.g. Robertson, 1985; Sherratt, 2001) and those which do not (e.g. Miller and Fincke, 1999) is that in the former case only, androchrome morphology may be constrained by the need to convincingly resemble males. Such constraints might arise from genetic correlation between the expression of male-like coloration and other aspects of the male phenotype, and/or from natural or sexual selection against androchromes that fail to resemble syntopic males. These predictions could be

tested by comparative morphometric analyses, with the expectation that in any given population androchromes are more similar to males than are gynochromes. Furthermore, if androchromes are selected to resemble males, then those androchromes that most closely resemble males should be less likely to be found copulating than poorer mimics.

Our study species, *Ischnura ramburii*, is known to have two distinct female morphs, one that resembles males and one that does not (Fig. 1). *Ischnura ramburii* is widespread throughout the New World ranging through most of the United States and Mexico, and as far south as Chile. In addition, *I. ramburii* was introduced to Hawaii sometime in the mid-1900s and was first recorded on Oahu and Kauai in 1973 (Zimmerman, 1948; Harwood, 1976). *Ischnura ramburii* has since spread throughout the island chain, and is now common on multiple islands (Polhemus and Asquith, 1996). We surveyed populations within *I. ramburii*'s native range, in Texas, and in the Hawaiian Islands, specifically on Oahu, Kauai, and Hawaii. Studying a species both in its native range and in an area where it has expanded into a novel environment allows us to investigate morphological variation in different evolutionary contexts. While both androchromes and gynochromes co-exist throughout *I. ramburii*'s native range, Hawaii lacked androchrome females as recently as the 1990s. Polhemus and Asquith (1996) state that although androchromes had been observed at extremely low frequency by Hilton (1989), they had not been seen in the archipelago in the intervening years. The range expansion of the species provides opportunities to determine how the colonization of new habitats and the resurgence of androchromy are associated with divergence in flight morphology, both within and between the sexes.

In this study, we used field-collected damselflies to conduct detailed and comprehensive investigations of wing morphology in a female-polymorphic odonate. Specifically, we tested: (1) whether morphs and/or the sexes differed in wing morphology and body size; (2) whether morphometric variation was predicted by mating status in the field; and (3) whether morphological traits varied geographically. If genetic correlations between androchromes and males, and/or selection for mimicry constrains androchrome morphology, we expect them to resemble males in more than just coloration. Furthermore, if androchromes are not constrained to mimic males, and both morphs are subject to negative frequency-dependent selection, we expect either no difference between androchromes and gynochromes, or no consistent pattern with respect to which morph most closely resembles the male. By sampling populations from several disjunct geographic areas, both within and outside of *I. ramburii*'s native range, we were also able to investigate regional differences in morphology and sexual dimorphism.

METHODS AND MATERIALS

Field collections and morphometric measurements

We surveyed three populations of *I. ramburii* on the island of Oahu and five populations on Kauai (Table 1). We obtained samples from a single population on the island of Hawaii, collected by Alexander Mikheyev in the city of Hilo. We also collected specimens from three populations in the state of Texas (Bastrop and Travis counties). All captured individuals were used to estimate morph frequencies. Due to damage during preservation, some wing images did not have all landmarks and were excluded from morphometric analysis. Thus our total sample sizes vary between number captured and fore- and hindwings. We analysed data for 300 forewings and 311 hindwings (Table 1).

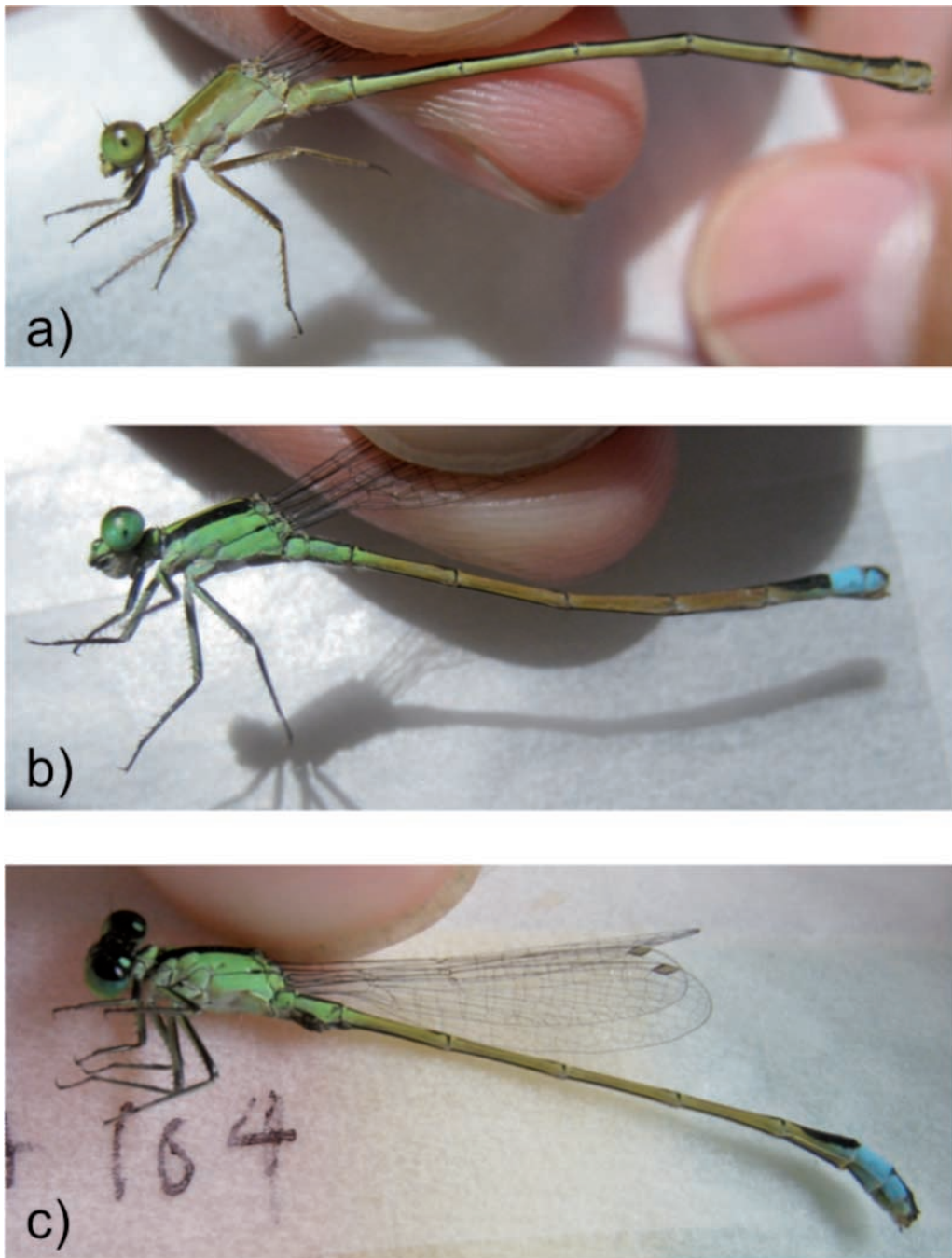


Fig. 1. (a) Female gynochrome, (b) female androchrome, and (c) male *Ischnura ramburii* showing typical sexually dimorphic coloration and female polymorphism.

Table 1. Site descriptions and GPS coordinates (NAD 1983) and sample sizes

Location	Region	Longitude	Latitude	# Androchrome (fore, hind)	# Gynochrome (fore, hind)	# Male (fore, hind)
Kalihi	Oahu	-157.88	21.34	8 (5, 7)	10 (9, 7)	17 (9, 11)
Manoa-Palolo	Oahu	-157.82	21.29	5 (4, 2)	13 (9, 9)	16 (9, 11)
Waimea Beach Park	Oahu	-158.06	21.64	8 (7, 8)	12 (11, 11)	20 (17, 19)
Anahola	Kauai	-159.31	22.14	1 (1, 1)	18 (12, 13)	18 (14, 16)
Hanalei	Kauai	-159.50	22.20	7 (7, 6)	13 (11, 11)	25 (21, 20)
Waimea Canyon Road	Kauai	-159.67	21.96	4 (2, 3)	18 (16, 18)	24 (20, 21)
Nawiliwili	Kauai	-159.35	21.96	4 (2, 4)	9 (6, 5)	20 (15, 16)
Kipu Falls	Kauai	-159.42	21.95	2 (2, 1)	4 (1, 1)	17 (13, 15)
Richardson's Ocean Park	Hawaii	-155.01	19.73	0 (0, 0)	11 (11, 10)	21 (15, 14)
Walnut Creek	Texas	-97.65	30.28	6 (4, 3)	12 (9, 8)	19 (16, 17)
Bastrop	Texas	-97.37	30.11	5 (3, 2)	14 (7, 9)	23 (12, 12)
Total				50 (37, 37)	134 (102, 102)	220 (161, 179)

Note: We report the total number of androchromes, gynochromes, and males captured at each site, followed in parentheses by number of fully digitized forewing and hindwing images.

All sites were visited 1–3 times during daylight hours, between 12.00 and 18.00 h. Individuals were collected using hand nets, placed in acetate collection envelopes, and brought to the laboratory where they were sacrificed by brief immersion in ethanol. Mating status for each individual was noted at the time of capture, as unmated (if collected alone) or mated (if collected either in the ‘tandem’ position, a precursor position to copulation, or in ‘wheel’, the copulatory pose), with the exception of the population on the Big Island of Hawaii, where these data were not collected. Although differentiating between individuals who were mating when captured provides a coarse measure of reproductive success, it provides a proxy of damselfly mating success that can be readily observed in the field (De Block and Stoks, 2007).

From each damselfly we removed all four wings, mounted these on glass slides, and then photographed the wings and bodies using a flatbed scanner at a resolution of 2400 dpi. We measured the total length of each individual’s abdomen using the ImageJ software package (Klingenberg, 2010). All damaged wings and bodies were excluded from our analyses. Twenty landmarks from each wing image were digitized using the TPSdig software program (Rohlf, 2003). Landmarks that could be reliably located on all wing images were selected to reflect overall wing shape (Bots *et al.*, 2009; Sadeghi and Adriaens, 2009). All landmarks were either vein intersections or points where veins reached the wing edge (Fig. 2).

Data analysis

We compared the frequency of the two female morphs across our sampled populations using a Pearson’s chi-squared (χ^2) test. We also tested whether the frequency of androchromes varied between mated and unmated females using a χ^2 test, with Yates’s correction for 2×2 tables. These analyses were run in R (R Development Core Team, 2011).

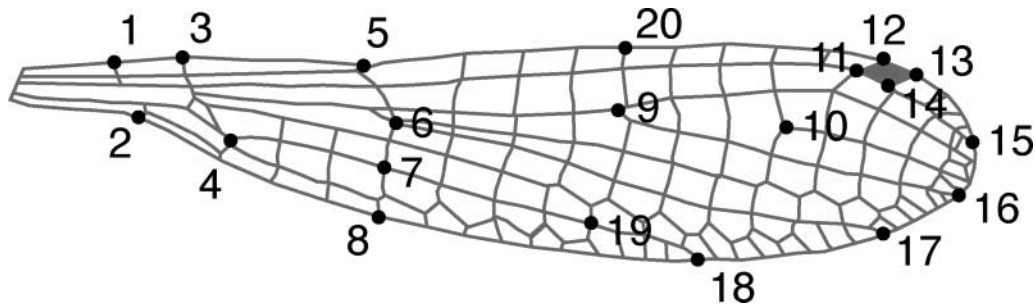


Fig. 2. Landmark locations used for morphometric analysis.

We calculated wing size metrics in R (R Development Core Team, 2011) using functions developed by Claude (2008). We estimated wing size as the sum of the distances of all landmarks to the centroid of the wing (Outomuro and Johansson, 2011). Wing length was measured as the distance between landmarks 1 and 15, while wing width was measured as the distance between landmarks 18 and 20 (Fig. 2). Aspect ratio was calculated as wing length/width. We compared wing sizes and abdomen lengths with analysis of variance (ANOVA) in SAS using Proc Mixed (Singer, 1998), with location (Oahu, Kauai, Hawaii, and Texas) and collection site within location as random effects, and sex, morph (nested within sex), and mating status as fixed effects. We tested for significant differences between pairs of populations using *post hoc* Tukey's Honestly Significant Difference (Tukey's HSD) tests, also in SAS.

To analyse differences in wing shape, we transformed the landmark values using partial generalized least squares Procrustes superimposition to remove size and orientation differences (Claude, 2008). This process allows for comparison of shape without the confounding effects of size differences. These analyses were performed only on right wings. We used multivariate analysis of variance (MANOVA) in SAS on the Procrustes normalized-transformed coordinates to assess whether sampling location, sex, morph, or mating status was associated with differences in wing shape. We used *P*-values calculated from Wilks' λ statistics. As previous researchers have noted (Bots *et al.*, 2009), forewings and hindwings differed markedly in shape, allowing us to identify mislabelled wings and discard those data (Fig. 3). To identify how populations and morphs differed in shape, we compared Procrustes coordinates in a canonical variance analysis (CVA) using the MorphoJ software package (Klingenberg, 2010).

RESULTS

Presence of Hawaiian androchromes confirmed

Androchromes and gynochromes were collected at both sites from Texas. In addition, we found both morphs present at all sites visited on Oahu and Kauai. This was somewhat surprising, given previous records indicating that only gynochrome females were to be found throughout the island chain. The coloration of these Hawaiian androchromes is male-like across the visible spectrum (unpublished spectrophotometer data), and indistinguishable from androchromes collected within their native range (in Texas).

Morph frequencies

We found that, overall, androchromes represented 27.1% of females, and this did not vary significantly across populations (Pearson's $\chi^2 = 15.1625$, d.f. = 10, $P = 0.1262$). In populations where mated females were collected, androchromes represented, on average, 32.5% of mated females. The proportion of androchromes did not differ between mated and unmated females (Pearson's $\chi^2 = 0.2352$, d.f. = 1, $P = 0.6277$).

Mating status

As we lacked data on mating status for the Big Island of Hawaii, we first ran all analyses excluding the individuals from that population. We detected no effects of mating status on any measured traits (Table 2). Therefore, we proceeded with our analyses on the full data set without considering wheel status as a possible predictor.

Abdomen length

Abdomen length varied geographically ($P = 0.0005$), and was largest on the Big Island of Hawaii. There was a significant difference in abdomen length between males and females ($P = 0.0445$), but not between morphs of females ($P = 0.2626$). There was also no relationship between abdomen length and mating status. However, as expected by allometry, there was a positive correlation between abdomen length and wing size, across males and females (ANCOVA adjusted $R^2 = 0.9$, $P < 0.0001$).

Wing size

Forewings and hindwings were significantly different in size (two-sample paired t -test: $P \ll 0.0001$) and shape (Fig. 3), thus we analysed them separately and present results for both. ANOVA revealed significant variation in wing size across regions (fore: $P = 0.0028$, hind: $P = 0.0039$). Individuals collected on the Big Island of Hawaii were larger than anywhere else (Fig. 4a). For both forewings and hindwings, we found highly significant differences ($P < 0.0001$ for both) in wing size between males and females (Table 2). Males' wings, like their bodies, were smaller than those of females. In addition, for forewings there was a significant effect of morph within sex as well ($P = 0.0088$). While both types of females had larger forewings than males, androchrome forewings were smaller than gynochrome forewings. This was true even when we excluded the Big Island of Hawaii, where there were no androchromes, from our analysis. This difference between the female morphs was not observed in hindwings ($P = 0.1841$).

Wing shape

ANOVA on aspect ratio (wing length/width) revealed that males and females differ in both forewings ($P = 0.0006$) and hindwings ($P = 0.0084$), with males having a higher aspect ratio (indicating more slender wings) in both cases. Female morphs did not differ in aspect ratio. Aspect ratio also differed across regions ($P = 0.0006$ for forewings, $P = 0.0084$ for hindwings; Fig. 4b) but not for locations sampled within the same region. Multivariate analysis of variance (MANOVA) on Procrustes-transformed coordinates showed that male

Table 2. Results of ANOVA for size differences in total abdomen length, forewing and hindwing centroid size, and forewing and hindwing aspect ratio

	Effect	d.f.	Error d.f.	<i>F</i> -value	<i>P</i> -value
Abdomen length	Region	3	7.1495	22.82	0.0005
	Location (nested in Region)	7	284	7.34	< 0.0001
	Sex	1	284	4.07	0.0445
	Morph (nested in Sex)	1	284	1.26	0.2626
	Region × Sex interaction	1	284	0.62	0.6009
	<i>Mating status</i>	<i>1</i>	<i>259</i>	<i>2.11</i>	<i>0.1480</i>
Forewing size	Region	3	7.1315	13.01	0.0028
	Location (nested in Region)	7	284	8.35	< 0.0001
	Sex	1	284	340.07	< 0.0001
	Morph (nested in Sex)	1	284	6.96	0.0088
	Region × Sex interaction	1	284	0.69	0.5612
	<i>Mating status</i>	<i>1</i>	<i>259</i>	<i>2.91</i>	<i>0.0890</i>
Hindwing size	Region	7	7.2179	11.53	0.0039
	Location (nested in Region)	3	295	8.29	< 0.0001
	Sex	1	295	289.14	< 0.0001
	Morph (nested in Sex)	1	295	1.77	0.1841
	Region × Sex interaction	1	295	0.65	0.5830
	<i>Mating status</i>	<i>1</i>	<i>272</i>	<i>0.21</i>	<i>0.6434</i>
Forewing aspect ratio	Region	7	8.2729	17.87	0.0006
	Location (nested in Region)	3	284	0.89	0.5111
	Sex	1	284	7.67	0.0060
	Morph (nested in Sex)	1	284	0.34	0.5602
	Region × Sex interaction	1	284	3.39	0.0186
	<i>Mating status</i>	<i>1</i>	<i>259</i>	<i>0.00</i>	<i>0.9517</i>
Hindwing aspect ratio	Region	7	8.201	7.92	0.0084
	Location (nested in Region)	3	295	1.55	0.1491
	Sex	1	295	8.96	0.0030
	Morph (nested in Sex)	1	295	0.07	0.7856
	Region × Sex interaction	1	295	3.56	0.0148
	<i>Mating status</i>	<i>1</i>	<i>272</i>	<i>0.04</i>	<i>0.8402</i>

Note: Significant effects are indicated in **bold**. Region (Hawaii, Kauai, Oahu, and Texas) and location (nested within Region) were treated as random effects. All other effects were fixed. We detected no effects of mating status on any measured traits (reported in *italics*). Thus we ran the full model excluding this term, and report those results.

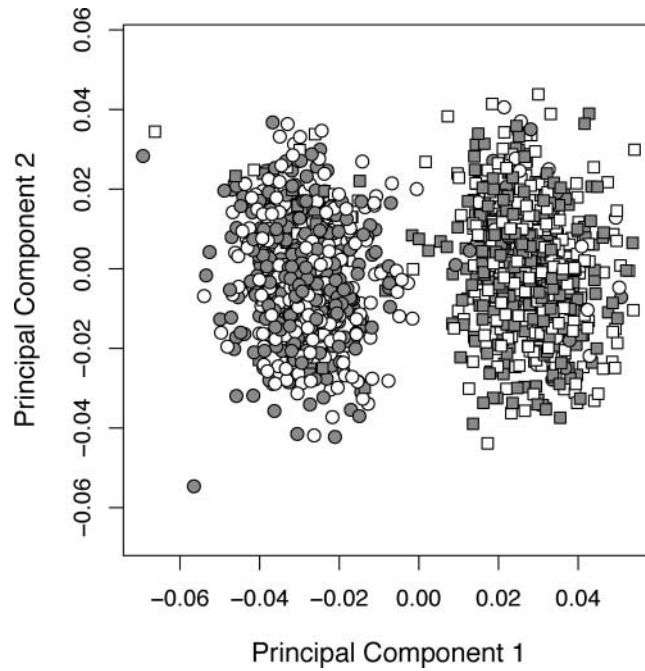


Fig. 3. Results of principal components analysis of Procrustes-transformed coordinates illustrating the distinct shapes of forewings and hindwings (circles and squares respectively), and the lack of differentiation between left and right wings (solid and empty respectively).

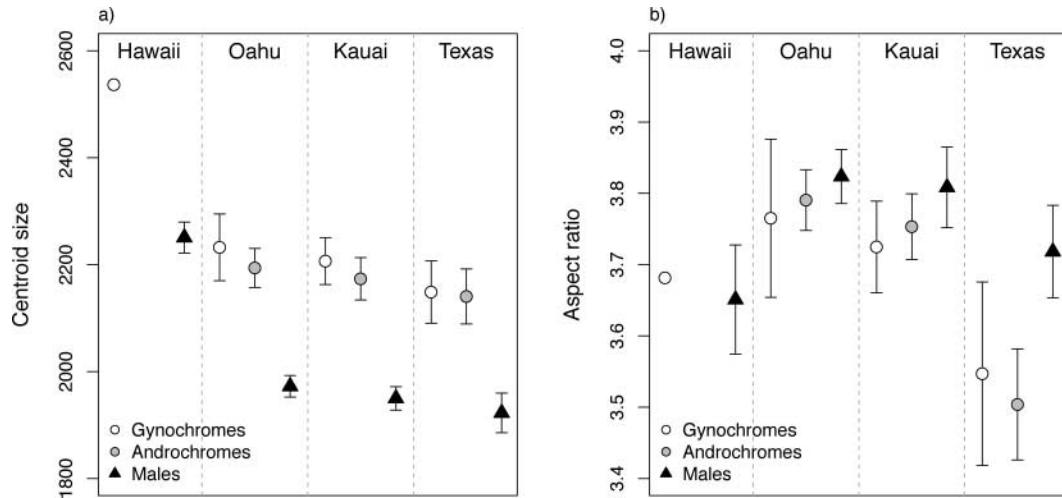


Fig. 4. Variation in (a) forewing centroid size and (b) aspect ratio across regions. Gynochromes are indicated in white, androchromes in grey, and males in black. Error bars represent 95% confidence intervals around the mean.

Table 3. Results of Procrustes MANOVA for shape differences

	Effect	Estimate	Num d.f.	Den d.f.	F-value	P-value
Forewings	Region	0.07	120	734.9	8.55	< 0.0001
	Location (nested in Region)	0.15	280	1704.8	1.94	< 0.0001
	Sex	0.34	40	245	12.04	< 0.0001
	Morph (nested in Sex)	0.91	40	245	0.62	0.9636
	Region × Sex interaction	0.55	120	734.9	1.33	0.0156
	<i>Mating status</i>	<i>0.81</i>	<i>40</i>	<i>220</i>	<i>1.24</i>	<i>0.1702</i>
Hindwings	Region	0.08	120	767.85	8.20	< 0.0001
	Location (nested in Region)	0.15	280	1780.7	1.98	< 0.0001
	Sex	0.47	40	256	7.32	< 0.0001
	Morph (nested in Sex)	0.9	40	256	0.67	0.9331
	Region × Sex interaction	0.56	120	767.85	1.37	0.0083
	<i>Mating status</i>	<i>0.81</i>	<i>40</i>	<i>233</i>	<i>1.36</i>	<i>0.0831</i>

Note: Significant effects are indicated in **bold**. Region (Hawaii, Kauai, Oahu, and Texas) and location (nested within Region) were treated as random effects. All other effects were fixed. We detected no effects of mating status on any measured traits (reported in *italics*). Thus we ran the full model excluding this term, and report those results.

wings varied significantly across geographical regions ($P < 0.0001$ for forewings, $P < 0.0001$ for hindwings; Table 3). Males were also significantly different from both androchrome and gynochrome females in fore- and hindwing shape ($P < 0.0001$ for forewings, $P < 0.001$ for hindwings), but the female morphs did not differ in wing shape ($P = 0.884$ for forewings, $P = 0.96$ for hindwings). The magnitude and direction of the difference between males and females varied across our four sampled regions: Oahu, Kauai, Hawaii, and Texas (aspect ratio: forewing, $P = 0.0186$; hindwing, $P = 0.0148$; MANOVA on shape: forewing, $P = 0.0156$; hindwing, $P = 0.0083$).

The CVA findings mirrored our Procrustes MANOVA (Table 4, Fig. 5). Male forewings were different in shape from female forewings (Procrustes distance between males and gynochromes = 0.0163, $P < 0.0001$; Procrustes distance between males and androchromes = 0.0147, $P < 0.0001$), but forewing shapes did not differ between female morphs (Procrustes distance = 0.0047, $P = 0.4077$). The first canonical variate (CV1) explained 94.7% of the variation among groups in forewings, while CV2 explained 5.26%. Permutation tests revealed that while males differ in shape from females, androchromes and gynochromes are not significantly different from each other (Table 4). Hindwings showed identical patterns, with CV1 explaining 93.2% of the variation among groups and CV2 explaining 6.76%. Male hindwings were shaped differently than those of both female morphs (Procrustes distance between males and gynochromes = 0.0172, $P < 0.0001$; Procrustes distance between males and androchromes = 0.0156, $P < 0.0001$), but no significant differences in shape were observed between the two female morphs (Procrustes distance between androchromes and gynochromes = 0.0031, $P = 0.9666$).

A separate CVA comparing the four sampling regions revealed significant shape differences between all sampling regions in hindwings (Table 5), and between all regions

Table 4. Results of canonical variate analysis for shape differences among males, androchromes, and gynochromes

	Androchromes	Gynochromes	Males
Androchromes		0.0047	0.0147
Gynochromes	0.4077		0.0163
Males	<0.0001	<0.0001	

Note: Upper diagonal elements are the pairwise Procrustes distances between groups. Lower diagonal elements are the *P*-values from permutation tests.

Table 5. Results of canonical variate analysis for shape difference across regions (Hawaiian Islands and Texas)

	Hawaii	Kauai	Oahu	Texas
Hawaii		0.0225	0.0198	0.019
Kauai	<0.0001		0.0047	0.011
Oahu	<0.0001	0.1155		0.011
Texas	<0.0001	<0.0001	<0.0001	

Note: Upper diagonal elements are the pairwise Procrustes distances between groups. Lower diagonal elements are the *P*-values from permutation tests.

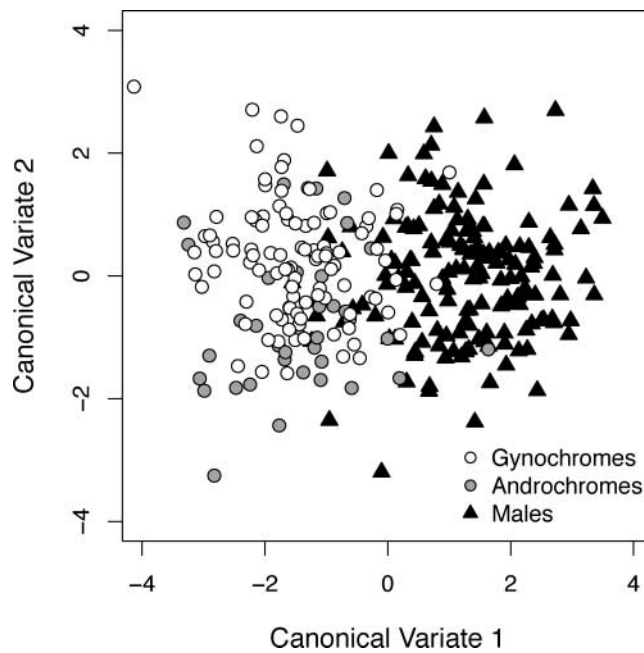


Fig. 5. Canonical variate analysis comparing the wing shape of males with that of the two female morphs.

except Oahu and Kauai in forewings (results not shown). Differences between the Big Island of Hawaii and the remainder of the locations were mainly reflected along CV1 for both forewings and hindwings. Furthermore, the Texas populations mainly diverged from the other three regions along the axis of CV2 in both forewings and hindwings. CV1 explained 62.6% of variation among islands in forewing shape, while CV2 explained a further 30.0%. Among hindwings, CV1 explained 61.7% of the variation across islands, while CV2 explained an additional 29.1%.

DISCUSSION

After confirming the presence of androchrome *I. ramburii* females in Hawaii, we investigated size and shape differences within and between the sexes. There was strong sexual dimorphism across all our sampled populations. Males had smaller bodies than females and possessed smaller wings, which were also more slender than those of females. Although androchrome *I. ramburii* females may appear male-like in their spectral patterning and some aspects of their behaviour (Robertson, 1985; Van Gossom, 2001; Sirot *et al.*, 2003; but see Iserbyt and Van Gossom, 2009), our morphometric analyses further revealed that they largely resemble gynochrome females in their wing shape. However, in forewing size, androchrome flight morphology did diverge from gynochromes, in the direction of males (Fig. 4). These patterns were consistent across studied populations, despite geographic differences in shape and size, and what may have been strong bottleneck and novel selection pressures following introduction to the Hawaiian Islands. We did not find any morphological correlates of mating status, but we had low power to detect these differences due to the paucity of individuals captured in mating postures.

Sexual dimorphism and maintenance of female polymorphisms

We found strong evidence for sexual dimorphism in all measured aspects of *I. ramburii* flight morphology. This sexual dimorphism confirms that differences between the sexes impose requirements on flight morphology, as has been demonstrated in other damselfly species (e.g. Moore, 1990; Anholt, 1997; Bots *et al.*, 2009). However, the direction and magnitude of wing shape differences between males and females varied geographically as in Abbott and Gosden (2009), suggesting that sexual dimorphism may be influenced by more than just reproductive roles.

In contrast with some other systems [*I. elegans* (Abbott and Gosden, 2009); *Enallagma cyathigerium* (Bots *et al.*, 2009)], the flight apparatus of *I. ramburii* females was very similar between the two morphs. This may indicate that natural selection on flight morphology for female-specific requirements constrains the ability of female damselflies to mimic males. Females are physically larger than male *I. ramburii*. And like their gynochrome counterparts, androchrome females must still carry out female-specific reproductive tasks (such as egg laying) that may impose specific flight requirements. Although some studies have shown that androchromes mimic male behaviour, which may select for male-like flight morphology (e.g. Andrés *et al.*, 2002; Sirot *et al.*, 2003), the lack of physical differences among female morphs is consistent with at least some cases in which conspecific female morphs did not differ in behaviour (Iserbyt and Van Gossom, 2009). However, Robertson (1985) describes *I. ramburii* androchromatic females mimicking male behaviour.

However, we did detect differences in forewing size between androchromes and gynochromes that are consistent with mimicry-based hypotheses. While the forewing size of androchromes is more similar to that of gynochromes than males, androchromes' wing size is smaller than that of gynochromes; and in the direction of male forewing size (Fig. 4). This finding agrees somewhat with that of Bots *et al.* (2009), who found that androchrome *E. cyathigerium* were distinct from gynochromes in several wing traits and were consistently more male-like. While these findings support selection for mimicry, several other mechanisms could explain the male–androchrome resemblance we report. For example, differences in conspicuousness to visually orienting predators may exert divergent selection on manoeuvrability and speed. If male-like patterning is more (or less) detectable, this should result in wing similarities between androchromes and males. Alternatively, perhaps the expression of male-like coloration imposes morphological convergence on other aspects of the male phenotype. Consistent with this scenario, Abbott and Svensson (2010) found that in the congeneric damselfly, *Ischnura elegans*, androchromes had higher intersexual genetic correlations than gynochromes.

Damselflies have well-developed visual systems (Briscoe and Chittka, 2001) and use a variety of cues, ranging from colour to behaviour, to make mate-choice decisions (Hammers *et al.*, 2009; Van Gossum *et al.*, 2011). Therefore, the relative importance of effective mimicry along each of these axes is unclear, and requires further experimental research.

Morphology and mating status

Mimicry-dependent hypotheses proposed to explain the maintenance of female polymorphism suggest that androchromes may be selected for by a relative reduction in male harassment, while carrying the potential cost of remaining unmated, and thus predict that androchromes should be mated at lower rates than gynochromes (Van Gossum, 2001; Van Gossum *et al.*, 2011). Frequency-dependent hypotheses predict that androchromes and gynochromes should be mated at rates determined by their relative frequencies (Miller and Fincke, 1999). Across our sampled sites we found that the ratio of androchromes to gynochromes did not differ between mated and unmated females. Although this is consistent with frequency-based hypotheses, the ratio of androchromes to gynochromes also did not vary significantly among sampled sites, which precludes us from making any strong conclusions about the relationship between mating status and morph.

Geographic variation

The geographic history of androchrome *I. ramburii* in Hawaii lends an interesting backdrop to our conclusions. The apparent resurgence of androchromes between the mid-1990s and 2008 suggests selection for the female colour polymorphism in *I. ramburii*. Although the genetic basis of the polymorphism is unknown for *I. ramburii*, in other damselfly species in which it has been investigated the trait appears to follow a simple Mendelian inheritance pattern of an autosomal locus with sex-linked expression (Van Gossum *et al.*, 2011). This would allow for an increase in the number of androchromes under certain selection regimes, as long as the allele was initially present at some, albeit low, frequency in the population. Many of the hypotheses proposed to explain male mimicry require population densities, or male-to-female sex ratios, high enough that male harassment is deleterious for females (Sherratt, 2001). While we lack records for historical population

sizes in Hawaii, immediately following introduction population sizes would have been low, and they were high when we surveyed in 2008. Increasing population densities may have generated increased selection for androchromes over this time period.

The most anomalous population we sampled, with respect to both morphology and morph frequency, was on the Big Island of Hawaii. All *I. ramburii* there were significantly larger than those sampled in all other locations, across both sexes. Notably, the sampled locality, as well as most of the island (E.J. Gering, unpublished), lacks androchromes. It is possible that bottlenecks created during range expansion underlie both female monomorphism and morphological divergence (Iserbyt *et al.*, 2010), but this would be surprising given the commonalities between the other Hawaiian populations and the native Texas populations. The lack of androchromes may be partially explained if population densities, or male-to-female sex ratios, are low enough there that there is not selection on females to avoid male harassment. Alternatively, the allele for female polymorphism may have been lost due to drift in the colonization of that island, and has not yet been reintroduced via dispersal from other polymorphic localities (Iserbyt *et al.*, 2010). The nearest island to the Big Island is Maui, 35 km away. Watts *et al.* (2007) showed that distances between populations as short as 10 km can be barriers to dispersal in the damselfly *Coenagrion mercuriale*, and open ocean crossings are likely even more challenging than the overland areas they studied. Nonetheless, human-mediated dispersal is entirely possible. Our ability to speculate as to the roles of bottlenecks and selection in the morphological divergence of Big Island individuals from other sampled populations is limited by our sampling only a single population there. Thus, while Big Island *I. ramburii* are physically larger and its females appear to be monomorphic, demonstrating a common underlying cause requires further investigation.

We were surprised to find more variation among the introduced populations in the Hawaiian Islands than between these populations and the ones collected from Texas, in the heart of *I. ramburii*'s native range. This suggests that there may have been rapid evolution of wing size and shape on the Big Island of Hawaii. Alternatively, the introduced populations may be descended from distinct introductions, perhaps from disparate native populations that vary morphologically. However, descent from a single introduction is also possible given that invasive Odonates are capable of rapid morphological evolution (Hernández-L. *et al.*, 2010). The degree to which androchrome females resemble males may differ depending on the frequency of males (models) in a given population, providing another source of geographic variation in wing morphologies (Iserbyt and Van Gossum, 2011). More thorough sampling across geography would allow us to disentangle these hypotheses in a way that is not possible with our current data.

CONCLUSIONS

Androchrome and gynochrome females of *I. ramburii* differ from males in flight morphology, as measured by wing size, wing shape, and aspect ratio. Although we found broad overlap between androchromes and gynochromes in flight morphology, the forewings of androchrome females are more male-like in size than those of gynochromes. This is potentially due to selection for male-like behaviour, either to avoid harassment via male mimicry, or to fit into a more male-like ecological niche. In addition, we found that androchrome females, which were nearly absent from Hawaiian populations in the mid-1990s, were common in Oahu and Kauai by 2008. This may have been driven by shifts

in selection for androchromy as *I. ramburii* population densities increased, following their introduction and establishment.

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