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CAUSES AND CONSEQUENCES OF COLOR POLYMORPHISM IN RAMBUR'S FORKTAIL (*ISCHNURA RAMBURII)*

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CAUSES AND CONSEQUENCES OF COLOR POLYMORPHISM IN RAMBUR'S FORKTAIL (*ISCHNURA RAMBURII*)

by

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Dedication

For three strong women:

Abigail Sorenson, Evelyn Gering, and Susan Bock.

I am living, I remember you

-Marie Howe

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CAUSES AND CONSEQUENCES OF COLOR POLYMORPHISM IN RAMBUR’S FORKTAIL (*ISCHNURA RAMBURII)*

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The University of Texas at Austin, 2013

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Variation in male and female forms occurs in countless animal taxa, and has fascinated evolutionary biologists since Darwin and Wallace. The underpinnings of male variation have been elucidated in diverse groups; less is known about the selective forces that diversify female forms in nature. Female-polymorphic damselflies provide ideal systems in which to study how female variation evolves. Color polymorphic damselflies typically contain one female morph that resembles the male (“andromorph”) and one or more alternative morphs with distinctive coloration (“gynomorphs” or “heteromorphs”). My thesis draws upon the unique context of a biological invasion to elucidate factors that promote and maintain this variation in female color.

Empirical work in my dissertation is focused upon Rambur’s Forktail (*Ischnura ramburii),* a species native to the Americas that invaded Hawaii in the 1970s. I first examine whether female color morphs diverge in mating rates or other reproductive traits within the native and invasive range, to see whether such traits might affect morph frequency dynamics in the invasion context (Chapter 2). Next, I test whether variation in selective regimes, both across female development and among populations, predicts variation in andromorph coloration (Chapter 3). Upon finding andromorphs to follow predictions of mimicry theory, I ask whether andromorph presence might result in increased male-male interaction rates, due to sex recognition errors (Chapter 4). Finally, I document recent, rapid evolution of andromorphy within Hawaii populations, and conduct mesocosm experiments to test the potential for density- and frequency-dependent selection to promote and maintain color polymorphism.

Results indicate 1) andromorphs may benefit from reduced mating, but male-like morphology may also incur reproductive constraints; 2) andromorph color variation accords with mimicry theory: andromorphs resemble syntopic males, and resemblance is maximized after reproductive onset; 3) male-male interactions increase in the presence of andromorphs, to male detriment; 4) gynomorphs are subject to negative-frequency-dependence in high-density populations, which may have driven the rapid evolution of andromorphy in Hawaii following introduction to the islands. These findings offer new insights into multiple mechanisms by which color polymorphism can arise and be maintained within native and invasive contexts.

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Chapter 1: An introduction to female-limited color polymorphism in the Odonata

FEMALE COLOR POLYMORPHISM OCCURS IN DIVERSE ANIMAL CLADES

Biologists since Darwin and Wallace have looked to intraspecific variation in male and female forms to understand how sex differences evolve. These efforts provide strong support for Darwin's idea that mate competition drives male trait evolution (Shuster 2009). Female-limited trait variation is less well understood due to historic emphases on male traits, and also the paucity of systems in which genetic variation in female morphology, physiology and/or behavior can be readily discerned. The dearth of models for female-limited polymorphism does not indicate scarcity, nor a lack of biological significance; it is a byproduct of the aforementioned biases towards the study of male traits, and also the sometimes limited apparency of female variability. By way of example, lab manipulations of polyandry in *Drosophila melanogaster* show that mating conflicts can promote rapid evolution of female resistance to manipulation by male sperm accessory proteins (Holland and Rice 1998). Variation in female *D. melanogaster* resistance, however, leaves no visible fingerprint on female morphology, would be challenging to identify and measure in nature. While difficult to observe and quantify, the diversification of female traits via sexual conflict is predicted to have profound consequences for ecology and evolution. It has been linked with speciation theory (Gavrilets 2004), and can create complex feedbacks between population demography and fitness (Arnqvist and Rowe 2005). These can, in turn, influence both trait evolution, and population persistence (Kokko and Rankin 2006).

Color polymorphisms provide powerful systems for studying how variation is maintained within species (Gray and McKinnon 2007), and female-limited color polymorphisms offer ideal systems in which to study how female trait variation evolves. In diverse animal groups, from hummingbirds (Bleiweiss 1992) to butterflies (e.g. Kunte 2009), some females resemble males (“andromorphs”), whereas others are chromatically dimorphic (“gynomorphs” or “heteromorphs”). The causes and consequences of this polymorphism remain an active area of research, with the Odonata (dragonflies and damselflies) comprising one of its best-studied exemplars. Damselfly color polymorphism has a simple genetic basis in each of five species examined (Johnson 1964, 1966, Cordero 1990, Andrés and Cordero 1999, Sánchez-Guillén et al. 2005), which makes it comparatively easy to compare fitness components between morphs, to link morph frequencies with variation in the social or physical environment, and to compare theoretical and empirical predictions about morph frequencies’ spatiotemporal dynamics.

BENEFITS AND COSTS OF MALE MIMICRY

More than a dozen verbal models have been forwarded to explain damselfly polychromatism (see Sirot et al. 2003). The best known of these is Robertson’s Male Mimicry Hypothesis (1985), which proposes that andromorphs’ resemblance to male conspecifics reduces unsolicited copulation attempts. Robertson proposed that this benefit of male mimicry is balanced by costs of expressing the male phenotype. As an example of balancing costs of andromoprhy, he cited predation risk and heterospecific mating attempts resulting from species recognition errors. Many other putative costs can also be imagined, which can be broadly divided into three categories: physiological costs (e.g. cost of acquiring and mobilizing pigments), ecological costs (e.g. costs of increased conspicuousness to prey), and genetic costs (e.g. suboptimal developmental programs arising from selection for protandry). Evidence for physiological costs was recently reported in *Nahelennia Irene* (Iserbyt et al. 2012), in which color morphs appear to tradeoff immune function (phenyloxidase activity) and flight musculature. In another study, intralocus genetic conflict was proposed to explain developmental differences between female color morphs in *Ischnura elegans*. Andromorphs' development, which was more rapid than gynomorphs', was hypothesized to result from selection on pathways shared by andomorphs and males, which could be under selection for male protandry (Abbott and Svensson 2005). Genetic conflicts may also explain morphological differences between morphs (Gosden and Svensson 2009), if traits selected for in males constrain female fitness (Gering et al. Chapter 2).

It seems unlikely that a simple balance of costs and benefits can explain the persistence of polychromatism throughout the Odonata. Costs of any sort might fluctuate across the vast ranges of many polymorphic species, vary over evolutionary time, and differ across species that diverge in mating systems, habitats, and other fundamental components of life history. Without any frequency-dependent mechanisms at play, variability in the cost:benefit ratio of andromorphy could be expected to produce fluctuations in morph frequencies, and possibly also give rise to monomorphic populations. Yet most polymorphic damselflies harbor both andromorphs and gynomorphs throughout their ranges (Fincke et al. 2005). More decisive evidence of frequency-dependent selection derives from findings that morph frequency variation within species is best explained by demographic variables (rather han by physical environments or biotic communities; e.g. Van Gossum et al. 2008, Gering Chapter 4). This correlation might be predicted to result from intralocus sexual conflict between andromorphs and males, a possibility that will require genomic analyses to properly test. While this hypothesis may prove correct, it is worth mentioning that andromorphs do not appear wholly constrained to resemble males apart from reproductively selected traits. In *I*. ramburii, some aspects of andromorph morphology are intermediate between gynomorphs' and males' (Gering Chapter 2), but female morphs overwhelming resemble one another much more closely than either resembles males (e.g. McTavish et al. 2012)

Another challenge to Robertson's male mimicry hypothesis is that the costs and benefits of andromorphy may change overt evolutionary time. Once multiple color morphs evolve, other traits could become linked with color and diverge between morphs (Forsman *et al*. 2008). This could facilitate divergence in any number of traits that partition available niche space (and are linked to color), but are not necessarily essential for polymorphism maintenance. Putative costs of andromorphy might also, upon close inspection, not affect fitness, or do so in unexpected ways. As one specific example, if male-like abdomen widths imposed by mimicry constrain an andromorph's egg traits, she may overcome this constraint by producing narrower eggs (Gering et al., Chapter 2).

FREQUENCY-DEPENDENT MATING CONFLICTS AND FEMALE POLYMORPHISM

Frequency-dependence (e.g. frequency-dependence in male-female interaction) provides a simple explanation for how polymorphism could be maintained in many species, and across large species ranges. Several frequency-dependent mechanisms for damselfly polymorphisms have been modeled quantitatively (e.g. Sherratt 2001, Fincke 2004, Svensson et al. 2005, Takahashi and Watanabe 2010). The first postulates that the ratio of andromorphs to males determines mimetic efficacy (Sherratt 2001); males learn to target male-like phenotypes when andromorphs are common, and leave them unmolested when they are rare. Other models (Fincke 2004, Takahashi and Watanabe 2010) allow that andromorphs are not mimics, and are never mistaken for males; males simply learn and harass the more common morph (Takahashi and Watanabe 2010). Svensson's model (2005) incorporates field-parameterized variation in female fecundity (both frequency-dependent and density-dependent) to show how demographic modulation of sexual conflict can maintain polymorphism; it does not specify the specific mechanisms through frequency-dependence emerges, and thus may or may not support Robertson's hypothesis of intersexual mimicry. Fincke's learned mate recognition model (2004) is similarly general in that it allows for a multiplicity of mechanisms to influence morph-specfic harassment rates, including mimetic efficacy, visual detectability, and male search image formation. Clearly, these are non-exclusive models, and some will be difficult to falsify. And of course, different mechanisms can operate between taxa, or even between populations, to promote polymorphism. The utility of each model will therefor depend upon whether an investigator aims to draw precise conclusions for a specific population, or gain general insight into polymorphism's unified causes and outcomes.

CAN ECOLOGICAL SELECTION PROMOTE POLYMORPHISM?

In female-polymorphic butterflies, frequency-dependent benefits of Batesian mimicry are commonly hypothesized to exert balancing selection on female color (Kunte 2009). Can natural selection also explain damselfly color polymorphisms? Cooper (2010) advanced this argument to explain the incidence of androchromatism in a native Hawaiian damselfly, *Megalagrion calliphya*. In this species, male-like coloration is associated with increased antioxidance, and an altitudinal gradient in female coloration may result from selection for UV- resistance within high-elevation habitats.

M. calliphya provides a fascinating example of ecologically-driven sexual dimorphism, but is unlikely to provide a general explanation for the incidence of polychromatism in damselflies. If abiotic variation exerted balancing selection on female color, relevant environmental parameters should be predictive of morph frequencies in space and time. As earlier stated, polymorphic species exhibit strikingly consistent frequencies across diverse ecological conflicts, and demography is more predictive of within-species variation than abiotic parameters.

Comparative approaches to female color polymorphism

One notable difference between *M. calliphya* and most color-polymorphic coenagrionids is that *M. calliphya* is territorial, whereas most color-polymorphic odonates exhibit scramble-competition mating systems. By defending territories in which females oviposit, male *M. calliphya* protect their reproductive partners from unsolicited copulation attempts, thus reducing the potential for mating rate conflicts.

Mating system variation has been overlooked as a reason for differences between findings concerning female polymorphism in different *Enallagma* and *Ischnura* species. Interestingly, even closely related polymorphic coenagrionids vary dramatically in mating systems. Some practice post-copulatory contact mate guarding, while males from congeners (such as *I. ramburii)* release females after copulation, leaving them vulnerable to harassment during ovipositioning (Abbott 2005). This variation could radically influence the susceptibilities of females to costly remating. Unfortunately mating system variation has not been mined to frame or test hypotheses for the incidence or frequency of polychromatism among coenagrionids.

Comparative approaches also present opportunities to gain insights into the implications of spectral differences between morphs and throughout development (Gering, Chapter 3). The ontogeny of color varies dramatically by species; in *Ischnura senegalensis,* andromorphs do not change color as adults (e.g. Takahashi and Watanabe 2012), in *I. heterostichtica* andromorphs lose their male resemblance upon reaching reproductive maturity, converging on gynomorph coloration (Huang and Reinhard 2012). In *I. ramburii* the reverse is true, and female morphs differ from one another in both pre- and post-reproductive coloration (Gering et al. Chapter 3), and in *I. elegans,* andromorphs are indistinguishable from gynomorphs until they reach reproductive maturity, and both pre-reproductive forms are subject to male harassment (Van Gossum et al. 2012). The ontogeny of male color is equally variable within this genus. This color variation could be used to better understand both how morphs differ in detectability to male conspecifics, predators, and prey, and to understand how male and female color ontogeny coevolve within a phylogenetic context.

In summary, there is ample evidence of a role for frequency-dependent sexual harassment in maintaining color polymorphism in the Odonata. However, species are likely to differ in the specific ways in which demography and other environmental factors interact with color polymorphism. Spectral properties, mating systems, and other factors that differ between species should be seen as opportunities to gain more detailed insights into mechanism that promote or constrain female trait divergence. These research aims will all require a robust phylogenetic framework in order to control for effects of shared ancestry on traits of interest.

As phylogenies are lacking, and foundations for interspecific comparisons remain incomplete, this thesis (Gering 2013) focuses on a single species. It provides some new insights into: a) potential constraints of male mimicry (Chapter 2), b) developmental and spatial variation in andromorph color (Chapter 3), c) effects of morph frequency on male-male interactions (Chapter 4) and d) potential effects of density and frequency on polymorphism evolution (Chapter 5). Some of this work (Chapter 4) reveals a consequence of female polychromatism that has not been previously considered, and likely matters for population dynamics. Other components (e.g. Chapter 5) provide unique corroboration of mechanisms, which have already been proposed to operate in other species. Ultimately these may both prove useful, as they can guide our approach to the study of polymorphisms in other contexts and systems.

Chapter 2: Male resemblance is associated with reduced mating rates and reproductive constraints in a female-polymorphic damselfly[1]

A woman's face is her work of fiction.
— Oscar Wilde

ABSTRACT

Female-limited color polymorphisms occur in many damselfly species, and their evolution is commonly ascribed to intersexual mating conflicts. However, ecological selection on linked traits could also promote color polymorphism. Here we examine *Ischnura ramburii* to determine how morphologies and egg traits differ between 'andromorph' females, which resemble males in color, and 'gynomorph' females, which do not. We hypothesized that if morphology differed between female morphs, andromorphs would most resemble males, due to constraints of effective mimicry. We also undertook a field survey and a mesocosm experiment to determine if andromorphs had reduced mating rates. We found that andromorphs: (i) had narrower abdomens, which more closely resembled those of males (ii) laid narrower eggs, perhaps due to constraints of abdomen width and (iii) mated less frequently than gynomorph counterparts in both field and experimental settings, as predicted by the male mimicry hypothesis. Only andromorphs showed a trade-off between clutch size and egg size, further indicative of constraints that might result from narrow abdomens. Our findings

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[1] Collaboration with Robert I. Etheredge (field assistant) and Molly E. Cummings (advised experimental design, analyses and writing)

corroborate a role for sexual conflict in female morph coexistence, but show that sexual conflict and ecological selection may interact in unexpected ways to maintain polymorphism. In the example of *I. ramburii*, male resemblance that reduces harassment may also constrain reproductive output.

INTRODUCTION

In recent years, there has been increasing interest in the evolutionary ecology of female color polymorphism. Several potential mechanisms have been proposed to maintain variation in female color; this study examines the two most widely discussed. First, female polymorphisms might be maintained by intersexual conflicts over mating rate. Female-polymorphic damselflies are well studied in this arena. They typically harbor a female morph with male-like appearance ("andromorph") and one or more morphs with alternative coloration and/or patterning ("gynomorph"). Andromorphs are believed to reduce sexual harassment by mimicking males (e.g. Robertson 1985), and/or due to frequency dependent mate recognition (e.g. Miller and Fincke 1999; reviewed in Van Gossum *et al*. 2008). The potential of andromorphy as a mimetic strategy is well evidenced by *I. ramburii,* the species for which the male mimicry hypothesis was first proposed. In this species, andromorphs closely resemble males (see Chapter 3); this resemblance sets *I. ramburii* apart from other well-studied female-polymorphic damselflies. The benefits of harassment avoidance have also been demonstrated in this taxon (Sirot and Brockmann 2001), and andromorphs were shown to have lower mating rates than gynomorphs in limited field studies (Robertson 1985).

More recent work, however, suggest that ecological selection on either coloration or linked traits might also play a role in the evolution of damselfly color polymorphisms. Studies from diverse color-polymorphic taxa have revealed that female conspecifics often vary in genetic compoents of reproductive strategy, which can be linked with color polymorphism (e.g. Sinervo *et al*. 2000; Roulin *et al*. 2003; Vercken *et al*. 2007, Mappes 2008). Life-history theory predicts that females face a trade-off between the number and quality of offspring they produce (e.g. Smith and Fretwell 1974). Balancing or fluctuating selection for large vs. high-quality clutches could thus promote color polymorphism via genetic linkage between maternal resource apportionment and color traits (e.g. Roughgarden 1971; Sinervo 2001; Sinervo *et al*. 2007). *I. ramburii* presents exciting opportunities to test this hypothesis because it inhabits a tremendous diversity of habitats, from desert ponds to tropical lagoons (Abbott 2005) that might favor divergent maternal resource apportionment strategies. Further, the introduction of this species into the Hawaiian Islands in the 1970s (Harwood 1976) created a unique context in which to test whether coloration and other (e.g. reproductive) traits remain integrated during the colonization of novel habitats. Further, the absence of andromorphs at detectable frequencies in Hawaii until the 1990s (see Chapter 5) allows for testing of whether the restablishment of polymorphism is associated with multivariate divergence between morphs. Both patterns would suggest either pleiotropic effects of the morph-determining gene and/or strong, convergent selection regimes across habitats and timeframes.

There are some indications that ecological factors, such as heterogeneity in the physical environment, might also promote female polymorphism (e.g. Cooper 2010). A recent study determined that andromorphs and gynomorphs of the female-polymorphic species *I. senegalensis* differ in the size and number of eggs produced, and concluded that trade-offs between these variables might lead to stable coexistence. While egg traits could also diverge via selection for reduced harassment (e.g. due to constraints incurred by male resemblance), this possibility has not been tested.

We examined the tradeoffs between egg morphology (egg size and shape) and clutch sizes of alternative female color morphs in Rambur's Forktail, *I. ramburii*. We also examined how these traits varied with maternal morphology and behavior, and their consequences for larval development. Our specific goals were to i) test whether female color and morphology predict mating rates, clutch sizes, and egg morphology, ii) examine consequences of egg trait divergence for larval development, and iii) test whether mating rate and/or other reproductive traits differed between morphs or across habitats and histories.

METHODS

Study Species and Sampling Localities

Ischnura ramburii is native throughout the Americas, and was introduced to Hawaii in the 1970s (Harwood 1976). This species belongs to the family Coenagrionidae, which contains >100 female-polymorphic species, which typically present multiple female color morphs throughout their native ranges. "Andromorph" females resemble males in coloration, and co-occur with one or more "gynomorph" (aka "heteromorph") females, which are spectrally dimorphic from male conspecifics. The genetic basis of this variation has been investigated in several species. In each studied coenagrionid, it was concluded that andromorphs and gynomorphs are differentiated by allelic variation at 1-2 autosomal loci with sex-limited expression (Johnson 1964, 1966, Cordero 1990, Andrés and Cordero 1999, Sánchez-Guillén et al. 2005, Takahashi *et al.* 2010).

Sampling efforts (described below) were part of ongoing studies into polymorphism dynamics within invasive populations; therefore our data are derived primarily from Hawaii populations. *Ischnura ramburii* was found to be widespread in low elevation habitats in shoreline vegetation of rivers, ponds, canals, and lagoons throughout the archipelago, and was never found at water features above 300m. General habitat descriptions are provided in Table 2.S1. To examine whether any differences observed between morphs are consistent across native and invasive populations, we also collected data on female mating rates and clutch sizes from native, Texas populations (Table 2.S1).

Analyses of Egg Traits

Clutches from 30 andromorphs and 129 gynomorphs collected in 2010 were used to investigate potential differences in egg traits between morphs. These clutches were obtained from female *Ischnura ramburii* collected in July and August from three Kauai Island populations, in which between 10% and 50% of females are andromorphs (Gering, unpublished data). Females were collected in the early afternoon, during peak mating activity for this species (Robertson 1985). Immediately after capture, females were placed in 8-ounce Tupperware containers equipped with Watman#2 filter paper, wetted with aged tap water, for 48 hours. The number of eggs laid over this short timeframe is considered to provide a useful, cross-sectional index of female fecundity (Svensson *et al*. 2005, but see Takahashi *et al*. 2010), since undisturbed females will oviposit all mature eggs within 24 hours of mating, and live for <2 weeks, on average, as adults (Corbet 1999, Sirot and Brockman 2001). An important assumption of this fitness proxy is that the number of offspring is predictive of the number of offspring that will survive to adulthood. This was recently confirmed in lab studies of the female-polymorphic species *Enallagma cyathigerum* (Bots *et al*. 2010), which is confamilial with *Ischnura,* and is also examined in the present study.

Whereas previous studies have used calipers and/or microscopy to measure both adult individuals and egg morphologies, we instead used an USB-powered flatbed scanner (Canon LIDE 200) to collect images of adult and egg morphologies, which provided an accurate and nondestructive method of acquiring data in field settings. Following the 48hr oviposition period, we quantified female morphology by scanning females at 1200dpi after positioning them on the scanner bed with microscope slides stacked upon their wings and on either side of the abdomen (to prevent movement and excessive pressure from the scanner lid). Scanned females were observed to walk, fly and behave normally upon return to their oviposition chambers. Also following the 48hr oviposition period, each filter paper was submerged in aged tap water for an additional three days, during which time eggs darken in color. Eggs were then counted non-destructively by scanning filter papers at 1200 dpi (Table 2.S1) and returned to their containers.

Ten-30 eggs from each clutch were measured and averaged to determine egg area and egg shape for each individual female. Morphological measurements of eggs and of females were obtained from scanned images using the program ImageJ (NIH). We measured the minimum width of the fourth abdominal segment (which is concave in shape) to assess abdomen width; length and flight apparatus were previously shown to be largely sexually dimorphic, but equivalent between morphs, in the sampling localities described here (McTavish et al. 2012).

We quantified egg shape in terms of circularity ($4\pi(\text{area}/\text{perimeter}^2)$); values approaching 1.0 thus describe increasingly round eggs. Egg sizes were measured as the number of pixels/egg, and clutch sizes were obtained by counting the total number of eggs in each scan, with counters blind to the identity of the contributing female. Because our early sampling efforts recovered mostly gynomorphs, few andromorph-laid clutches hatched before the field season's conclusion. We therefore used a subset of gynomorph clutches from a single population (Princeville, Kauai; n=44) to examine whether hatching time was predicted by egg morphology or clutch size. These clutches were examined each afternoon for 30 days to determine hatching dates; in each clutch the majority of eggs (80-100%) hatched within a few hours of one another. We also counted the total number of eggs that hatched from 22 of these clutches, and used this dataset to examine the relationship between clutch size and egg viability using logistic regression. Morph differences in egg shape, egg size, and clutch size were tested using mixed models, with morph type fitted as a predictor variable, and population effects were included where significant (additional details of model selection are provided below).

Analyses of Morphology (Abdomen Width), Clutch Size, and Mating Frequencies.

To compare mating rates, clutch sizes, and morphologies, we used a larger dataset that was collected between 2009-2010 from populations on the islands of Kauai, Oahu and Hawaii, and in Texas (n=226 andromorphs, 1012 gynomorphs; for sampling localities, see Table 2.S1). All sampling was carried out in July and August according to the protocols described above. Analyses of these data were restricted to females that were deemed mature based on the coloration and hardness of the cuticle and wings (Corbet 1999). Because each population was sampled in only one year, we did not include a year effect in our analyses.

We also present a pilot partition of the data presented later in this thesis (see Chapter 4, tests of frequency- and density-dependence in female performance) to see if males differed in per capita clasping of either morph. Male experience affects mate searching in a number of female-polymorphic coenagrionids (Miller and Fincke 1999, Takahashi and Watanabe 2009). Experiments were conducted with andromorphs at 50-87% frequency, under conditions described in Chapter 4. We expected that if frequency-dependent harassment alone accounts for polymorphism maintenance, then andromorphs in experimental populations should be harassed more than gynomorphs when their frequency exceeds an unknown threshold frequency. While this threshold need not be 50%, we expected andromorphs to be disadvantaged under the tested conditions (50-87% andromorph) as andromorphs are almost never the majority morph in native and invasive sampling localities (see Chapter 4, Fincke et al. 2005). In contrast, if andromorphs are harassed less than gynomorphs at even relatively high frequencies, this would corroborate Robertson's suggestion that costs of andromorphy (which may take many forms) balance the benefits of reduced harassment.

All analyses were conducted using the open-source software package R (R Core Development Team 2009). We first fit mixed models using restricted maximum likelihood methods implemented by the lme4 package (Bates and Maechler 2010), including population as a random effect in all cases where significant (see Table 2.S2). To determine the significance of population as a random effect, we used restricted maximum likelihood ratio tests (RLRTs) implemented by the RLRsim package (Scheipl et al. 2008). We then refit models using maximum likelihood methods, and confirmed the presence/absence of population-level variation by examining MCMC simulation results obtained from languageR (Baayen et al. 2008) to determine if random effect estimates included values of zero. Results from both methods were consistent in all cases. We used likelihood ratio tests (of models fit using maximum likelihood) to evaluate the significance of population as a random slope, as well as for all other model comparisons. In all cases, the best fitting model obtained by these methods was also the one favored by AIC and BIC criteria. Finally, we confirmed that our omission of population-level effects (where non-significant) did not change the fixed effects present in our most parsimonious models. We took this step because some have argued for the inclusion of population effects in all models, irrespective of statistical significance, whenever they are present in experimental designs. We modeled the abdomen widths of both males and females using morph type (male, andromorph, gynomorph) as a predictor variable; pairwise contrasts between treatment levels were then conducted with the function glht in the multcomp package (Hothorn *et al*. 2008). We then restricted analyses to females in order to investigate the interdependence between maternal morphology, clutch size and mating status. We modeled clutch size (a Gaussian response variable) as a function of three predictor variables (color morph, mating status, and interaction), and obtained parameter estimates for the best fitting model using MCMC simulation (n=10000 simulations) implemented in the languageR package (Baayen *et al*. 2008). To test effects of abdomen width on female mating status (a binomial response variable) we analyzed each morph separately using generalized mixed models.

RESULTS

Relationships Between Female Morphologies, Mating Rates, and Clutch Sizes.

Morphs and sexes differed in abdomen width; andromorphs had narrower, more male-like abdomens than gynomorphs (Figure 2.1; $p<0.001$ for each pairwise comparison), and were less likely to be found mating than gynomorphs (Figure 2.2; $p<0.01$). This pattern was not broken by any single well-sampled locality (Table 2.S2). In experimental populations housing andromorph majorities, clasping rates were higher for gynomorphs (see $z=2.433$; $P=0.015$). Overall, females with wider abdomens were more likely to be found mating (Figure 2.2), a pattern which was significant for gynomorphs ($p=0.02$) but not andromorphs ($p=0.37$), perhaps reflecting differences in sample size ($n=226$ andromorphs, $n=1012$ gynomorphs) and/or effect strength for the two groups. The best fitting model for clutch size revealed a positive regression for abdomen widths of both andromorphs and gynomorphs ($P=0.03$), but no morph*clutch size interaction ($x^2=0.28$, $p= 0.87$; Figure 2.3).

Tradeoffs Between Clutch Size and Egg Size

Morphs differed significantly in the relationship between fecundity and mean egg size ($x^2_{\text{colormorph * egg size}}=4.02$, $p=0.04$). Specifically, andromorph females exhibited a significant tradeoff between egg number and egg size, whereas gynomorphs did not (Figure 2.4). As expected, clutch size was positively correlated with the number of larvae hatching from a clutch ($F_{1,20}=77$; $r^2=0.79$; $p<0.001$), and the 95% confidence interval for this relationship bounded one to one (slope = 0.62-1.04). Thus, offspring returns from increased clutch sizes were not counterbalanced by reduced egg viability.

Morph Differences in Egg Traits.

Morphs did not differ significantly in either the size of eggs laid ($t_{\mathbf{38.24}}$=0.45, p=0.66) or in the number of eggs laid ($t_{48.95}$=0.17, p=0.86). However, morphs differed in egg shape; the eggs of andromorphs were more elongate than those of gynomorphs ($t_{\mathbf{43.02}}$=-2.21, p=0.033; Figure 2.5).

Effects of Egg Morphology on Development

Due to low capture rates for andromorphs in our early field sampling, we were not able to make quantitative comparisons of hatch rate and viability between morphs; we therefore used natural variation within gynomorphs to examine how egg morphology affects development. We found that larger eggs hatched more quickly than smaller eggs (t_{area}= -2.452, p=0.023; Figure 2.6). In contrast, gynomorphs' egg shapes did not influence time to hatching ($t_{circularity}$=-0.976, p=0.34; Figure 2.6), and their egg sizes were not significantly correlated with egg shape (t_{size}=-0.765, p=0.45).

The limited data that we obtained from andromorph clutches (n=4) indicated that morphs do not differ qualitatively in either rates of egg development (time until ≥10% eggs hatched =13.25±1.7days for andromorphs; 13.6±1.5days for gynomorphs), or egg viability (92±3% hatch success andromorph; 89±15% hatch success gynomorph).

DISCUSSION

Morph differences in reproductive traits

In *Ischnura ramburii*, we found that morphs differ in mating rates, with andromorphs mating less frequently than gynomorphs in both native and invasive populations. These results support the male mimicry hypothesis, which proposes that andromorphs resemble males in order to reduce unsolicited detection by mate searching males. We also found that alternative female color morphs differ in their body and egg morphologies. Specifically, andromorph females have both narrower abdomens and narrower eggs than gynomorph conspecifics (Figure 2.1, Figure 2.5). While *I. ramburii* morphs did not differ in the size or number of eggs laid, they differed in the relationship between these two variables. Only andromorphs exhibited a tradeoff between egg number and size, suggesting a reproductive constraint (Figure 2.4).

While difference in egg shapes between morphs might be subject to ecological selection, our results suggest they might instead reflect differences in morphological constraint imposed by sexual conflict. This idea has been suggested by previous studies of *I. elegans* (Abbott and Gosden 2009, Gosden and Svensson 2009), in which andromorph abdomen width is negatively associated with fecundity. Abdomen width has been shown to influence male mate recognition in damselflies (Gorb 1998), and in *I. elegans,* andromorphs showed a positive relationship between abdomen width and mating rate after controlling for other morphological variation (Gosden and Svensson 2009). A recent study also found that andromorphs have more male-like body shapes in populations where andromorphs are common, as predicted if requirements for effective deception (male mimicry) vary according to morph frequency, and male resemblance incurs reproductive penalties (Iserbyt *et al*. 2011). Our data reveal that these morphological differences may also impose constraints on egg shape.

If this hypothesis is correct, it is curious that in our study, the positive relationship between abdomen width and mating rates was significant only for gynomorphs. However, andromorphs may still incur greater penalties for increased abdomen width, since costs of male interactions can differ between morphs (Sirot and Brockman 2001, Gosden and Svensson 2009), and since deviation from male-typical morphology could incur pre-contact harassment costs, which were not measured in the present study.

Our results reinforce previous findings from *I. ramburii* and other taxa (e.g. Robertson 1985, Cordero *et al*. 1998, Gosden and Svensson 2007), in that andromorphs appear to have lower mating rates than gynomorphs (Figure 2.2). Yet whereas *I. elegans* andromorphs produced smaller clutches than gynomorphs (Svensson *et al*. 2005), we found no differences in clutch sizes between morphs in *I. ramburii*. The production of narrower eggs that we report here might allow *I. ramburii* andromorphs to produce large eggs and clutches, while maintaining any benefits (e.g. reduced harassment) accruing from narrow abdominal profiles. We note, however, that potential consequences of egg shape variation merit closer examination, especially given observed differences in development time in *I. elegans* (Abbott and Svensson 2005), and the potential for egg shape to affect dessication risk, oviposition substrate, and larval emergence (Corbet 1999).

While experimental tests of how female morphology affects male harassment are needed, the present study highlights the potential for morphological adaptation to sexual conflict to influence life-history strategy. Our findings thus have implications for life-history evolution beyond color polymorphic systems, given the ubiquity of sexual conflicts in nature (Arnqvist and Rowe 2005). Whereas resource competition has long been thought to shape life histories (Pianka 1970), morphological constraints, such as those imposed by predation (e.g. Congdon and Gibbons 1987) and tradeoffs between reproductive and flight performance (e.g. Berrigan 1991) can also mold female reproduction over evolutionary time. We suggest that reproductive tradeoffs associated with male avoidance may be a similarly important source of constraint, though such constraint will be harder to detect in the absence of linked color variation.

Consequences of egg traits for maternal fitness

Although egg and clutch sizes did not differ between *I. ramburii* morphs, both traits were highly variable among females, allowing us to test two previously assumed consequences for maternal fitness. First, we confirmed that the number of eggs laid in a clutch predicts the number of hatching offspring (Figure 2.6), providing support for the assumption that clutch size provides a meaningful fitness proxy within this group (e.g. Svensson *et al.* 2005, Gosden and Svensson 2009, but see Fincke 1986). Further support for this fitness proxy comes from a recent lab study of *Enallagma cyathigerum* in which clutch size predicted the number of offspring surviving to adulthood (Bots *et al.* 2010). In the latter study, the relationship between clutch size and maternal fitness was reduced at later stages of larval development, indicating that maternal and/or genetic effects also determine surviving offspring numbers.

One of many potential components of larval survival is egg size variation. Our study is the first to confirm the supposition that in *Ischnura,* egg size predicts the rate of pre-larval development (e.g. Takahashi and Watanabe 2010; Figure 2.6). This pattern has now been reported from many arthropods, including dragonflies (Hottenbacher and Koch 2006), and likely results from positive relationships between egg size and vitelline content. The egg size variation we discovered in *I. ramburii,* and attendant variation in development, likely has important consequences for maternal fitness in nature. Since damselflies commonly experience size-structured predation and cannibalism as larvae, both the timing of hatching and larval body size can influence survival and condition (Corbet 1999, Padeffke and Suhling 2003). In *I. senegalensis,* gynomorphs produce larger eggs and fewer eggs than andromorphs, a strategy that is predicted to evolve in stable environments where resource competition is high (Pianka 1970). We did not find egg size differences between morphs; hence balancing selection for r- and K-reproductive strategies does not appear to explain polychromatism throughout *Ischnura*. Nonetheless, our collective support for egg size and clutch size as female fitness components suggests that the morph differences in the allometry of these traits (Figure 2.4) could be important for morph coexistence in *I. ramburii*.

Comparative insight into female color polymorphism

The potential for color polymorphism to influence the ecological dynamics of animal populations (Forsman *et al*. 2008) and drive speciation (Gray and McKinnon 2007) were extensively reviewed in the recent literature. In both cases, linkages between coloration and coadapted traits were invoked as important components of population dynamics and divergence. As taxa diverge, the mutability of such linkage will likely influence both the distribution of polymorphism throughout a clade, and its ecological consequences for descendent species. Yet empirical studies of color polymorphism have largely focused on individual representatives of polymorphic clades.

By expanding upon analyses from multiple color polymorphic species (Table 2.2), and collecting data from both native and invasive populations, we obtained several insights into the potential underpinnings of female-limited polymorphism. Most importantly, it can now be shown that female coloration, morphology, and reproductive traits are not coupled in a concerted fashion across closely related species. For example, whereas *I. senegalensis* gynomorphs produce smaller clutches than conspecific andromorphs, this pattern was not observed in *I. ramburii*, and is reversed in *I. elegans*. Thus, no particular life-history strategy is coupled with male-like coloration and morphology, even among closely related taxa. The observed differences between damselfly species mirror results from studies of more distantly related taxa from other groups: female color morphs in the genus *Uta* have been shown to differ in maternal resource allocation (Sinervo 2001), but such differences were not detected in a recent study of a female-polymorphic *Anolis* species (Cox and Calsbeek 2011).

In contrast, *within* species differences between *I. ramburii* morphs are strikingly consistent between native and invasive populations, suggesting that similar factors could maintain morphs in both contexts. Elucidating which factor(s) maintain polymorphism in these contexts will require experimentation, but field observations and previous work (on *I. ramburii* and other species) implicates male-female interactions as playing a key role in maintaining alternative morphs. The success of invasive species is often attributed to the absence of enemies (preadotors and parasites) and competitors within non-native habitats, a phenomenon referred to as ecological release (see Keane and Crawley 2002). Intersexual conflict might be a particularly strong selective agency within invasion contexts, where both density increases and selection for increased dispersal could increase male-female encounters, and other selective forces are attenuated. We encourage further replication of these types of studies across related taxa, and within diverse ecological contexts to better understand how sexual conflict and ecological selection drive the evolution of female forms.

ACKNOWLEDGEMENTS

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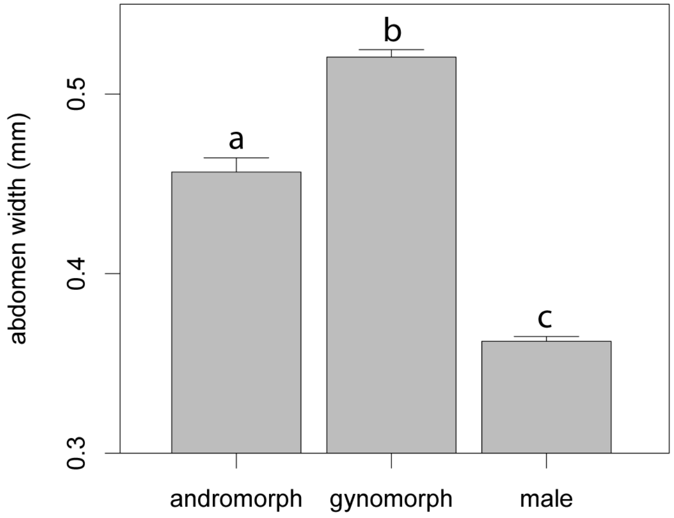

Figure 2.1 Widths of the 4th abdominal segments of males and the two alternative female color morphs that co-occur in *Ischnura ramburii*. Letters indicate significant differences between groups (p<0.001 for each pairwise comparison; see Table 2.S1).

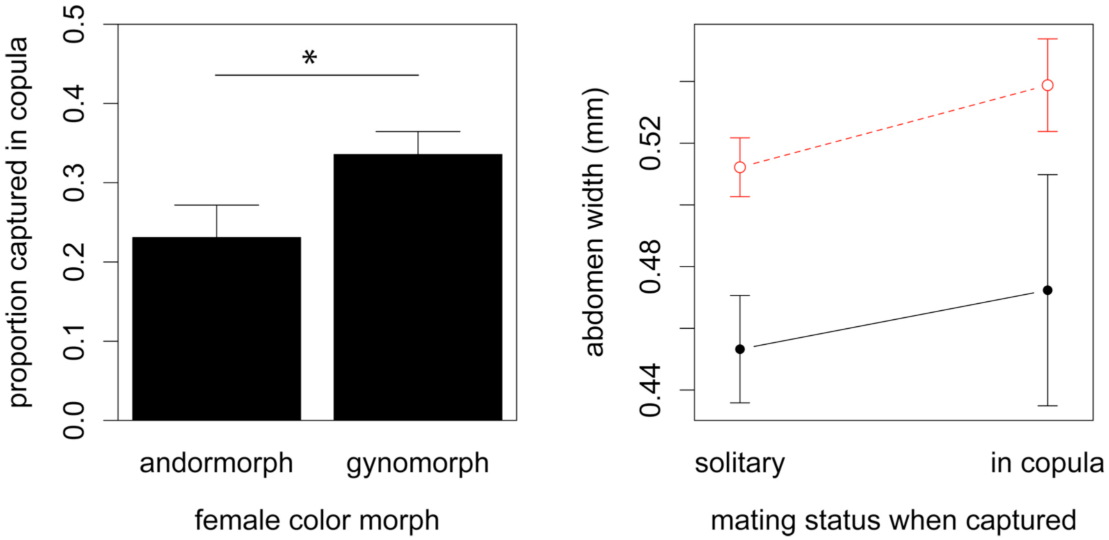

Figure 2.2 In *Ischnura ramburii*, andromorph females had lower mating rates than gynomorphs (left panel; z=3.12, p=0.002). Mating gynomorphs had larger body widths than non-mating counterparts (right panel; p<0.01; open circles, n=497), and a non-significant trend in the same direction was observed for andromorphs (right panel; p=0.37; closed circles, n=195). Error bars in each panel indicate 95% confidence intervals from analyses of pooled data.

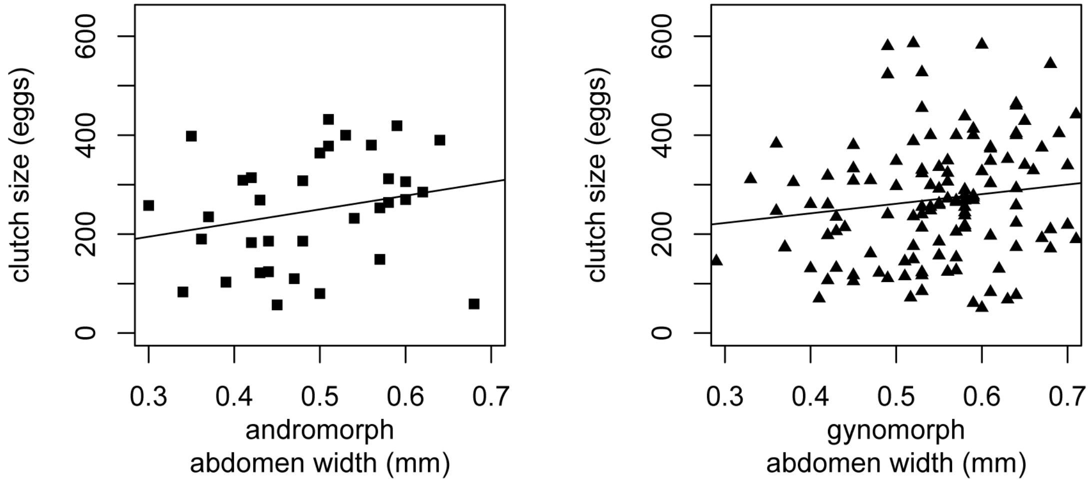

Figure 2.3 In *Ischnura ramburii*, both female color morphs (andromorphs and gynomorphs) exhibited weak but positive relationships between body width and clutch size (pMCMC=0.016). The lack of a statistical interaction between morph type and abdomen width (x^2=0.26, p=0.87) suggests that this relationship scales equally for both morphs.

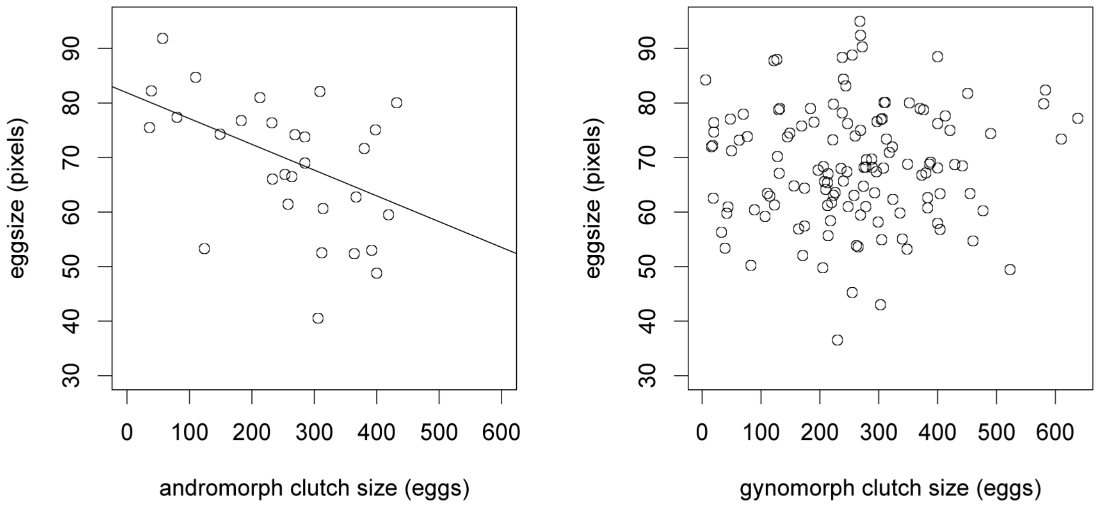

Figure 2.4 Clutch sizes are negatively correlated with egg size (pixels/1200dpi scan) among *I. ramburii* andromorph females (left panel, $p=0.023$, $r^2=0.17$). No correlation was detected among gynomorph females (right panel, $p=0.61$, $r^2=0.002$).

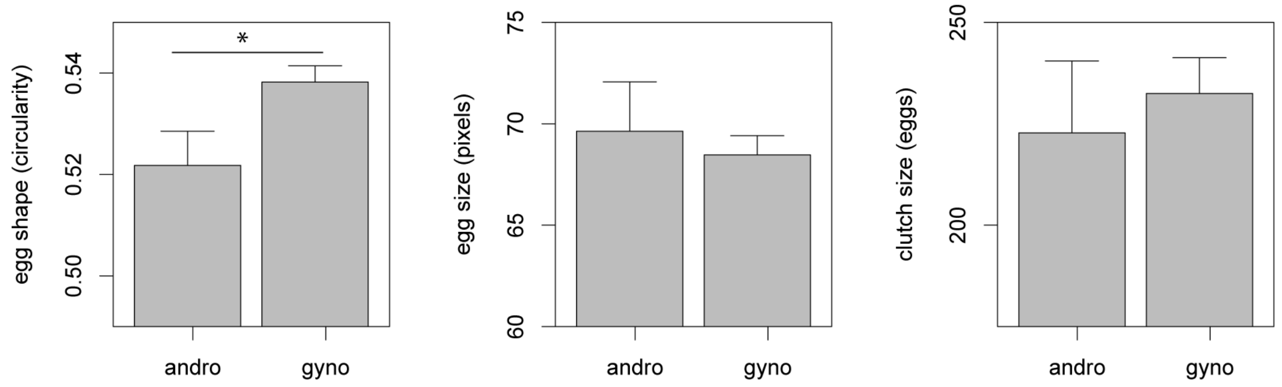

Figure 2.5 Means and standard errors of egg shapes (left panel), egg sizes (center panel), and clutch sizes (right panel) for alternative female color morphs from three Kauai populations of the damselfly *Ischnura ramburii*. Andromorphs and gynomorphs differed in egg shape (p=0.033), but not in egg size (p=0.66) or clutch size (p=0.86).

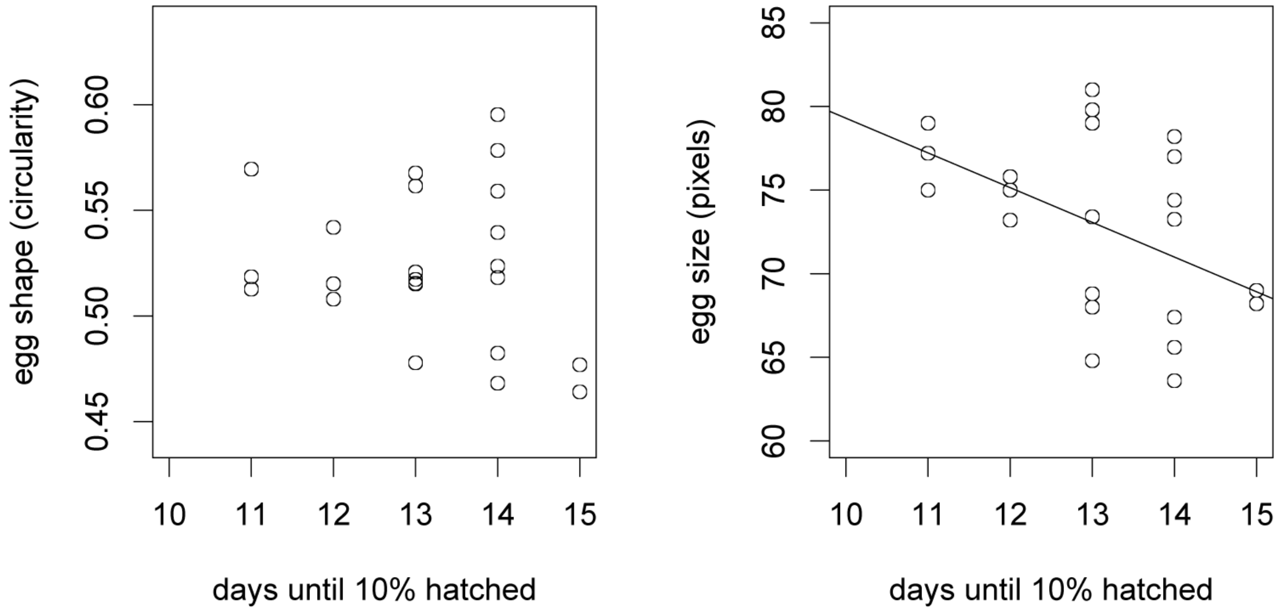

Figure 2.6 Hatching times for eggs laid by *Ischnura ramburii* gynomorphs were not predicted by egg shape (left panel; p=0.34, r^2=-0.04). Large eggs developed more rapidly than small ones (right panel; p=0.023, r^2=0.21).

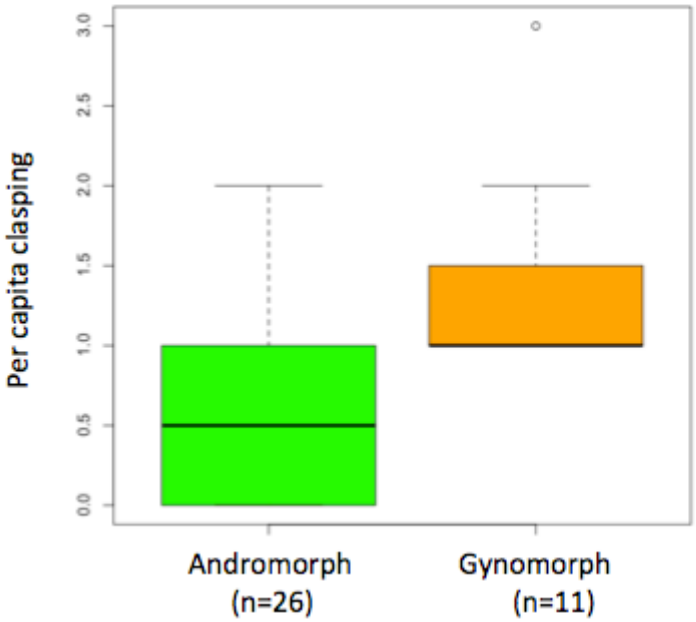

Figure 2.7 Rates at which males *I. ramburii* males clasped andromorphs and gynomorphs housed in experimental mesocosms. Frequencies of andromorphs (50% and 87%) in these experiments were higher than those found in the field.

Table 2.1 Relationships between egg traits, mating rates, female morphology and coloration in three *Ischnura* species. A=andromorph females, G=gynomorph females

taxon	Clutch size	Egg size	Egg Shape (circularity)[1]	Mating Frequency	Abdomen Width
Ischnura elegans[2,3,4]	G>A	?	?	G>A	G>A
Ischnura senegalensis[5,6]	A>G	G>A*	?	(Frequency-dependent)	?
Ischnura ramburii[7]	A=G	A=G	G>A	G>A	G>A

[1] circularity=4π(area/perimeter^2), [2] Cordero *et al*. 1998, [3] Svensson *et al*. 2005, [4] Gosden and Svensson 2007, [5] Takahashi and Watanabe 2009, [6] Takahashi and Watanabi 2010, [8] present study

Figure 2.S1 Example of scanned egg clutch from which morphological measurements were taken. A spacer was used to prevent scanner lid from crushing eggs.

Table 2.S1: Sampling localities and sample sizes used to examine relationships between maternal morphology, mating rates and clutch sizes of alternative female color morphs in *Ischnura ramburii*.

Region	Locality (year)	GPS	Andro In copula	Gyno In copula	Nandro fecundity	N gyno fecundity	N males	Habitat
Hawaii Island	POL (2009)	20°12'11"N 155°43'55"W	30	174	5	58	180	Wide and shallow watercourse in cattlefield, located within high walled, remote valley on the Hamekua (NE) coast.
Oahu Island	MPA (2010)	20°12'11"N 155°43'55"W	33	77	10	16	145	Rapid flowing stream with shallow standing pool in urbanized valley
Oahu Island	SLP (2010)	21°21'11"N 157°54'34"W	34	38	12	14	122	Large saline pond at ca. 200m above sea level in suburban area near Pearl Harbour
Kauai Island	MEN (2010)	21°56'44"N 159°23'10"W	10	90	2	16	136	Large remote reservoir within wildlife preserve at sea level near Lihue Kauai
Kauai Island	PRV (2010)	22°13'26"N 159°29'27"W	54	212	11	54	191	Ornamental ponds within condo community established ca 1995
Kauai Island	TAR (2009)	22°12'6.39"N 159°29'52"W	37	131	9	23	119	Tarot fields in Hanalei Valley, extremely wet and continuously farmed and flooded in rotational scheme since pre-invasion
Kauai Island	WCR (2009)	21°57'46"N 159°40'5"W	11	164	4	60	188	Pond on private land in arid scrub habitat along road to Waimea Canyon, no other standing water in the area, co-inhabited by small population of E.civile
Texas	BLP (2010)	30°11’19”N 97°27’86”W	30	42	30	42	36	Large Park on Bastrop lake administered by LCRA.
Texas	MLP (2010)	30°29’41”N 97°70’30”W	45	68	45	68	60	Recently constructed pond at Mueller redevelopment in the heart of Austin

Table 2.S2 Population-level variance components in best fitting models of *Ischnura ramburii* traits

Response Variable	Significant fixed effect(s)	Among population (=random) intercept?	Among population (=random) slope?
Abdomen width (Gaussian)	Morph (Male/Andro/Gyno)	**Yes** (RLRT = 99.66; $p < 0.001$)	**Yes** ($X^2 = 27.5$; $p < 0.001$)
Clutch size (Gaussian)	Abdomen width	**Yes** (RLRT = 4.74; $p = 0.011$)	**No** ($X^2 = 0.02$; $p = 0.99$)
Egg shape (Gaussian)[1]	Morph (Andro/Gyno)	**Yes** (RLRT = 13.59, $p < 0.001$)	**No** ($X^2 = 0.03$; $p = 0.99$)
Gynomorph mating status (Binomial)[1]	Abdomen width	**Yes** ($X^2 = 27.81$; $p<0.001$)	**No** ($X^2 = 3.7096$; $p = 0.08$)

[1] Analyses of egg size and of andromorph mating status did not reveal significant predictor variables.

Chapter 3: Age- and habitat-specific selective regimes predict color variation in male-mimicking females[2]

> *Men marry women with the hope they will never change. Women marry men with the hope they will change. Invariably they are both disappointed.*
>
> *— Albert Einstein*

ABSTRACT

Selection for intersexual mimicry is believed to maintain color polymorphism in diverse taxa, yet key predictions of mimicry theory remain untested in these groups. Here, we conduct novel tests of the male mimicry hypothesis, which postulates that 'andromorph' (male-like) female damselflies mimic male conspecifics in order to avoid unsolicited copulation attempts. First, we tested whether developmental color change in andromorph females maximizes their resemblance to males following reproductive onset, when both the potential for, and costs of male harassment are highest. Quantitative spectral analyses revealed that reproductive andromorphs resemble males in both color and brightness, whereas mature gynomorphs, and pre-reproductive females of both morphs, do not. Secondly, we tested whether geographic variation in andromorphs mirrors that of males. Consistent with male mimicry's predictions, we discovered that the coloration of males and of mature andromorphs covary across populations. In contrast, male color was not correlated with the color of mature females of an alternative morph ('gynomorph'), or with juvenile females of either morph. Together, our findings offer

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[2] collaboration with Molly E. Cummings (advised experimental design, analyses and writing)
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novel support for the hypothesis that andromorphs mimic males, and show how intersexual mimicry can be more completely understood by quantitative contrasts of model and mimic phenotypes, both among populations and across developmental stages.

INTRODUCTION

"Sex-limited" polymorphism refers to conspicuous trait variation that occurs within only one sex of a given species. One well-established example of sex-limited polymorphism is female mimicry by males (i.e. 'sneaker males'), which disguise their sexual identities in order to obtain covert access to mates (reviewed in Gross 1996, Brockmann 2001, Shuster and Wade 2003). While the ultimate causes of male-limited polymorphism have been thoroughly examined (e.g. Ryan et al. 1990, Shuster and Wade 1991, Endler 1992, Sinervo and Lively 1996), those underlying female-limited polymorphism have not been as widely studied, despite the occurrence of female polymorphism within diverse vertebrate and invertebrate clades (Kunte 2009). This deficit remains an important gap in evolutionary biology, since female polymorphisms can both influence ecological processes, and facilitate the diversification of traits and/or species (e.g. Gray and McKinnon 2007, Forsman et al. 2008, Svensson et al. 2009).

Female-polymorphic odonates (dragonflies and damselflies) have recently emerged as model systems for the study of female-limited polymorphism (Van Gossum et al. 2008). In many female-polymorphic odonates, selection for male mimicry is thought to diversify female color. Polymorphic odonates generally harbor one female morph with male-like coloration (the "andromorph"), which co-occurs alongside counterparts that have distinctive coloration and/or patterning (“gynomorphs”, or “heteromorphs”). The male mimicry hypothesis proposes that andromorph females mimic conspecific males in order to attenuate various costs that could arise from excessive male-female interaction (Robertson 1985). Although there are several behavioral and morphological studies supporting this hypothesis (e.g. Robertson 1985, Iserbyt et al. 2011), there have been surprisingly few quantitative assessments of the resemblance between male and andromorph coloration. Further, it remains unknown how this resemblance varies across populations and/or female reproductive states. Here we take advantage of significant developmental and geographic color variation in *Ischnura ramburii*, the species in which male mimicry was originally proposed, to test spectral predictions derived from the male mimicry hypothesis.

In *I. ramburii*, andromorphs undergo pronounced developmental color changes (e.g. Corbet 1999, Abbott 2005), and while the sexes will eventually become indistinguishable to the human eye, they can be easily discriminated from one another upon their emergence from the larval stage (Figure 3.1). First, we tested whether andromorphs resembled males most closely after becoming reproductive, when they begin frequenting male-dense pondside habitats to lay eggs. Harassment avoidance would be advantageous at this stage since male interactions with previously mated females can negatively impact female fitness (Sirot and Brockman 2001, Gosden and Svensson 2009, Takahashi et al. 2010).

Second, we tested whether extensive geographic variation in female *I. ramburii* coloration (across 10 populations in Texas and Hawaii) is predicted by geographic variation in male coloration. Specifically, we predicted that coloration of mature andromorphs would covary with syntopic (locally-occurring) males, while that of gynomorphs and non-reproductive andromorphs would not. Taken together, our joint aims provide complimentary tests of basic mimicry theory, which holds that 1) effective mimicry requires resemblance to locally occurring models, and 2) mimics should most closely resemble models under conditions (e.g. life stages) that select for effective deception.

METHODS

Field sampling

Ischnura ramburii is native throughout much of the Americas, and was introduced to Hawaii in the 1970s (Harwood 1976). Sampling efforts were part of ongoing studies into polymorphism dynamics within native (Texas) and invasive (Hawaiian) populations; thus both regions were represented in our sampling (Table 3.S1), which occurred in July and August 2010-2011. All collections were made in the early afternoon, during peak mating activity for this species (Robertson 1985). Collections at each site were undertaken on multiple, staggered days to reduce daily variation in population makeup introduced by weather and/or stochastic fluctuations in age structure. The mating status of each individual was recorded upon capture, and individuals were also assigned to one of two age classes (‘young’; i.e. recently eclosed, vs. ‘mature’) based upon wing hardness and wear (Corbet 1999).

All females were placed in 8-ounce oviposition chambers equipped with damp Watman#2 filter paper and left undisturbed for 48 hours; reproductively mature females will lay all matured eggs shortly after capture (e.g. Svensson et al. 2005, Gering, personal observations), and continue to produce and lay eggs each day in captivity (Sirot and Brockmann 2001). After 48 hours, we removed females from the oviposition chambers and submerged filter papers in aged tap water for three days; during this time eggs darken and can thereafter be unambiguously identified by visual inspection.

Spectral data collection:

Individuals were selected ad hoc for spectral analysis; chosen individuals were measured with the scanner and/or spectrometer within 2 hours of capture. Sixty five individuals were processed with both methods to allow comparisons of resultant spectral indexes. Spectrophotometry data were collected with an EPP200C UV-VIS spectrometer and R400-7 reflectance probe (StellarNet Inc., Tampa, FL) illuminated by a pulsed xenon lamp (PX-2, Ocean Optics) controlled by a custom-built trigger. Spectralon white standard measurements were taken at each recording session. The averaged reflectance of 4 scans from the thorax of each individual was used for subsequent analyses. RGB scans were obtained by placing females on a Canon LIDE 200 scanner with three microscope slides stacked atop their wings, and additional slides surrounding their bodies to prevent excessive pressure and/or movement during scanning. A 50% gray card was included in each scan to evaluate the continuity of exposure across scans, and landmarks on the thorax were used to create a bounding box for measurement of thoracic color (Figure 3.S1), using the public domain program Imagej (U.S. National Institutes of Health;

http://rsbweb.nih.gov/ij/). This region was selected because it does not include melanic stripes, which vary in size and intensity between individuals, and would therefore complicate our analyses and predictions.

Analyses:

All analyses were conducted using the open-source software package R (R Core Development Team 2009). To permit comparisons between scanner data and spectroscopy, reflectance measurements obtained by spectroscopy were divided into bins comprising short wavelength (300-500nm), middle wavelength (500-600nm) and long wavelength (600-700nm) components, which were compared to RGB scanner data (described below) using regression. Since male color peaks within the middle range of the spectrum (Figure 3.2), we also calculated a “green index” defined as the ratio of reflectance between 500-550nm to the total reflectance measured (the integrated sum of reflectance, at 1 nm increments, from 300-700nm). We compared both the green index and total brightness (300-700nm) for all developmental and genetic morphs (young male, mature male, young andromorph, mature andromorph, young gynomorph, mature andromorph) using Bonferonni adjusted p-values to permit testing of multiple spectral indexes. We included population as a random effect in all analyses because it was present in our sampling design. Because we were also interested in determining whether populations differed in spectral properties, we determined the significance of population effects post hoc, by examining whether confidence intervals of highest probability density (HPD-95) from MCMC simulations (n=1000) bounded zero, using the pvals.fnc function in the languageR package.

To compare coloration of males and females across populations, between mating and non-mating andromorphs, and between reproductive and pre-reproductive andromorphs, we also collected scanned images from a larger sample of individuals (Table 3.S1), and calculated the mean pixel saturations of red, green and blue color channels (henceforth 'R', 'G', 'B') within the region of interest for each individual. Two metrics were extracted from the scanner datasets for further analyses of color: total brightness (R+G+B; "scanned brightness") and a 'green index' (G/(R+G+B; "scanned green index"). We then compared population means for males with those of other groups using linear regression. Finally, we calculated the difference between each andromorph's scanned brightness and scanned green index, and the average values of males from the female's sampling population. We then used general linear models to determine whether mating status and/or reproductive status predicted the degree of resemblance between andromorphs and syntopic males.

RESULTS

Mature andromorphs resemble males:

Both brightness and green index differed significantly across age classes of each female morph, but young and mature males did not differ significantly from one another (Table 3.1); males were therefore pooled for subsequent analyses. Using our spectral reflectance dataset, we found that spectra of males and mature andromorphs exhibited similar shapes across populations, with pronounced peaks at 525nm (Figure 3.2).

Whereas males and mature andromorphs were not significantly different in spectral indexes, male brightness and green index were distinctive from gynomorphs and young andromorphs in each of two well-sampled populations (Figure 3.2).

Male and mature andromorph color covary across populations:

Spectrophotometry also revealed that populations differ in both brightness and green index: after accounting for age differences (as fixed effects, where significant), MCMC simulations found significant population-level variation for males, andromorphs, and gynomorphs. We therefore used the larger RGB dataset to compare population means for spectral indexes of males and females. In order to assess the utility of RGB data, we first analyzed the relationship between spectroscopy and RGB measures. Spectral indexes obtained from the two methods were highly correlated (r^2 ranged from 0.53 to 0.89 for R, G, B, brightness, and green index; Figure 3.S2), confirming that the two measures yield comparable measurements of these indexes. We also used Imagej to analyze gray standards from a random sample of scans taken in 2010 (n=34) and 2011 (n=34). This allowed us to assess how much variation might be introduced by differences in the illumination of individual scans. In fact, we recovered very little variation in R, G and B values of gray standards (Figure 3.S3); indicating that the scans provide a reliable and repeatable methods of obtaining information about both color and brightness.

RGB analyses revealed significant population differences in brightness and green index (Figure 3.3). As predicted by male mimicry, both brightness and green indexes were positively correlated between andromorphs and males across populations, and these relationships did not have significant intercepts (brightness: t=3.319, $p<0.004$,

$t_{intercept}$=1.883, $p_{intercept}$=0.08; green index t=3.68, slope=0.75±0.17(sd); t=4.29, $p<0.0005$; $t_{intercept}$=1.36; $p_{intercept}$=0.20). No significant relationships were found between green indexes of males and young andromorphs (t=0.30; p=0.76), young gynomorphs (t=-1.15; p=0.27), or mature gynomorphs (t=1.01; p=0.33). Nor were significant relationships apparent between scanned male brightness and that of young andromorphs (t=0.31; p=0.76), young gynomorphs (t=1.67, p=0.12), or mature gynomorphs (t=1.87, p=0.08).

Reproductive andromorphs most closely resemble males:

When analyses were restricted to mature andromorphs, andromorph resemblance to male green index was predicted by both mating status ($F_{1,136}$= 6.16, $p<0.02$) and reproductive state ($F_{1,136}$= 40.56, $p<0.001$). Mature andromorph resemblance to male brightness was also predicted by mating status ($F_{1,136}$= 9.47, $p<0.01$) and reproductive state ($F_{1,136}$= 11.97, $p<0.001$). Compared to non-mating and pre-reproductive counterparts, mating and post-mating, reproductive females were more similar to males. Estimates for residual color scores of reproductive andromorphs met the predictions of male mimicry (Figure 3.4); 95% confidence intervals for both brightness and green indexes of these females did not diverge significantly from syntopic males.

DISCUSSION

This is the first geographical study of color variation in a female-polymorphic damselfly; it revealed that the coloration of male *Ischnura ramburii* varies among populations, and thus poses a potential challenge for intersexual mimicry by females.

However, andromorph coloration also varies spatially, such that andromorphs resemble syntopic (locally-occurring) male counterparts (Figure 3.4). This correspondence between male and female color is consistent with the male mimicry hypothesis, which proposes that andromorphs mimic males in order to avoid unsolicited sexual interactions.

Our study also linked female color ontogeny with reproductive status. We predicted mimicry would be most beneficial after reproductive onset because 1) ovipositioning requires females to enter the exposed, pondside environment where mate-searching males are aggregated at high densities, 2) previously-mated females may gain less from subsequent male interactions than (sperm-limited) virgin counterparts. This prediction was met by data from field-captured andromorphs in both the native and invasive range of *I. ramburii,* and did not hold for gynomorphs (Figures 2 and 3), and by the finding that spectral indexes of previously-mated andromorphs were statistically indistinguishable from males, whereas younger andromorphs exhibited sexual dimorphism (Figure 4.4).

Our spectral comparisons (Figure 3.2) also indicate that age and short wavelength reflectance are correlated for both female *I. ramburii* morphs. Age-structured color variation may therefore be an important determinant of the frequency and cost of harassment from male conspecifics. There is evidence that frequency-dependent mate recognition arises from males preferentially cueing on prevailing local morph(s) (e.g. Takahashi et al.2010). Male learning and attendant morph-specific harassment might therefore depend upon encounter rates with different developmental morphs, as well as with alternative genetic morphs (Sirot et al. 2003).

Interestingly, the only other study to explore the relationship between andromorph color and reproductive status (Huang and Reinhard 2012) found a pattern opposite of our findings for *I. ramburii*. In an Australian damselfly*, I. heterosticta:* andromorphs first resemble males (before reaching reproductive maturity), and then become indistinguishable from gynomorph females upon becoming reproductive. Male *I. heterosticta* do not attend to pre-reproductive andromorphs, suggesting the possibility that male mimicry protects andromorphs from harassment as juveniles. Another distinction between the two species is that *I. heterosticta* gynomorphs and andromorphs are indistiniguishable after reaching reproductive maturity, and thus may be equally subject to male harassment. In contrast, the mating rates of mature *I. ramburii* andromorphs are lower than gynomorphs in both field and lab studies (Gering, unpublished data), a pattern also seen in other *Ischnura* species (e.g. *I. elegans*, Gosden et al. 2010), where mature andromorphs resemble males. In these species, morphs can also differ in the fitness consequences of multiple matings (Gosden et al. 2010), with gynomorphs being more tolerant of matings surpassing their fitness optima. Together, these findings suggest that differences between *ramburii and heterosticta* color ontogeny may represent differences in the tolerance of mature vs. immature females to male harassment. It would thus be useful to compare the conspicuousness of ontogenetic morphs to the male visual system; this would provide insight into whether the apparewntly conspicuous pre-reproductive morphs in *ramburii* may be signaling their reproductive state to males.

Alternatively or additionally, differences in color ontogeny may derive from differences in the consequences of microhabitat partitioning for mature vs. immature andromorphs, and/or reflect different stages of a coevolutionary race with male conspecifics. Whether or not these specific factors can explain species differences in color ontogeny, it is clear that phylogeny, selection and conflict might play important and interactive roles in the evolution of andromorph phenotypes, and that it will be important to examine how these forces act across different life stages.

Several alternative mechanisms may underlie the geographic correspondence between male and andromorph color phenotypes we discovered. Environmental feedbacks, such as spatial variation in pigment availability, could have parallel effects on both sexes. Alternatively, spatially varying natural selection may exert similar pressure on male and female phenotypes across habitats (e.g. Cooper 2010). It is also possible that a common set of genes influences the mature coloration of both males and andromorphs, and that selection and/or drift at these loci necessarily produces correlated change in both sexes (e.g. Abbott and Svensson 2010). While this may prove to be the case for color, it is already known that andromorphs are not genetically contrained to resemble males for other morphological traits: wing morphologies an body lengths of both female morphs in *I. ramburii* are highly similar, and different from males (McTavish et al. 2012).

Finally, selection for effective male mimicry could explain the correspondence between male and mature andromorph color (Figures 3 and 4). This would also explain apparent differences in the mating frequencies of *I. ramburii* andromorphs and gynomorphs in the field (Gering et al. in review). Further, if males are under diversifying selection to avoid male-male harassment (Sherratt 2001), then differences in andromorph-male resemblance across populations might reflect different stages of a sexually-antagonistic coevolutionary race. Determining which mechanism(s) explain variation in andromorph-male resemblance will require experimental studies, and/or elucidation of the genetic and environmental determinates of damselfly colors, which are poorly understood.

Within populations, human observers report close resemblance between the coloration of male and andromorph *I. ramburii* (Robertson 1985, personal observations). This is not the case for many female-polymorphic damselflies (e.g. Abbott 2011, Takahashi et al. 2012), in which andromorphs only partially resemble males in color and/or patterning. In other well-studied species, e.g. *Ischnura elegans,* males undergo more pronounced ontogenetic color changes than occurs in *I. ramburii*, and thus provide a wider array of models for intersexual mimics to emulate. This species is also distinctive from *I. ramburii* in that immature andromorphs and heteromorphs cannot be distinguished from one another, which would complicate both predictions for, and interpretations of andromorph color ontogeny. And in *Ischnura senegalensis,* males and andromorphs, which are spectrally dimorphic, do not change colors following emergence (Takahashi et al. 2012). Some of these distinctions likely contributed to the central role of *I. ramburii* in the formulation of Robertson's male mimicry hypothesis (1985), and may indicate differences in the function of female color variation across species. Thus, "mixed evidence" for male mimicry derived from different species may not be problematic for the hypothesis, but rather reflective of variation in the degree to which sexual deception selects on female color and behavior. Comparative studies of damselfly color ontogeny are currently lacking, and will likely prove instructive and exciting.

Finally, we point out the value of quantitative assessments of the resemblance between models and putative intersexual mimics, which has relevance for both male and female mimicry. Such comparisons are common in systems in which apostatic selection is thought to promote Batesian mimicry (e.g. Darst and Cummings 2006), but, surprisingly, have seldom been undertaken within hypothesized exemplars of intersexual mimicry. Iserbyt et al. (2011) recently showed that andromorph body shapes resembled males more closely in populations where andromorphs are common relative to gynomorphs, and suggested this pattern could arise from frequency-dependent selection for mimetic fidelity. Our analyses of coloration provide a valuable further test of the male mimicry hypothesis; unlike body shape, andromorph and male color are dramatically distinctive upon emergence from the larval stage (Figure 3.1), and converge only after female maturation.

We found basic predictions of mimicry theory were upheld in comparisons of *I. ramburii* color across populations and developmental stages, and will be excited to see whether this pattern holds for other systems. Comparisons between models and putative intersexual mimics will be useful not only for testing whether mimicry explains standing patterns of phenotypic variation, but also for elucidating how selection for mimicry might interact with other selective agencies to promote or constrain diversity.

Table 3.1 Analyses of developmental variation in spectrophotometric indexes of *I. ramburii* males and alternative female color morphs from Texas and Hawaii populations.

	df_{age}	p_{age}
male green index	2,76	0.56
andromorph green index	4,55	**<0.0001**
gynomorph green index	10,85	**<0.0001**
male brightness	2,76	0.33
andromorph brightness	4,55	**<0.01**
gynomorph brightness	10,85	**<0.0001**

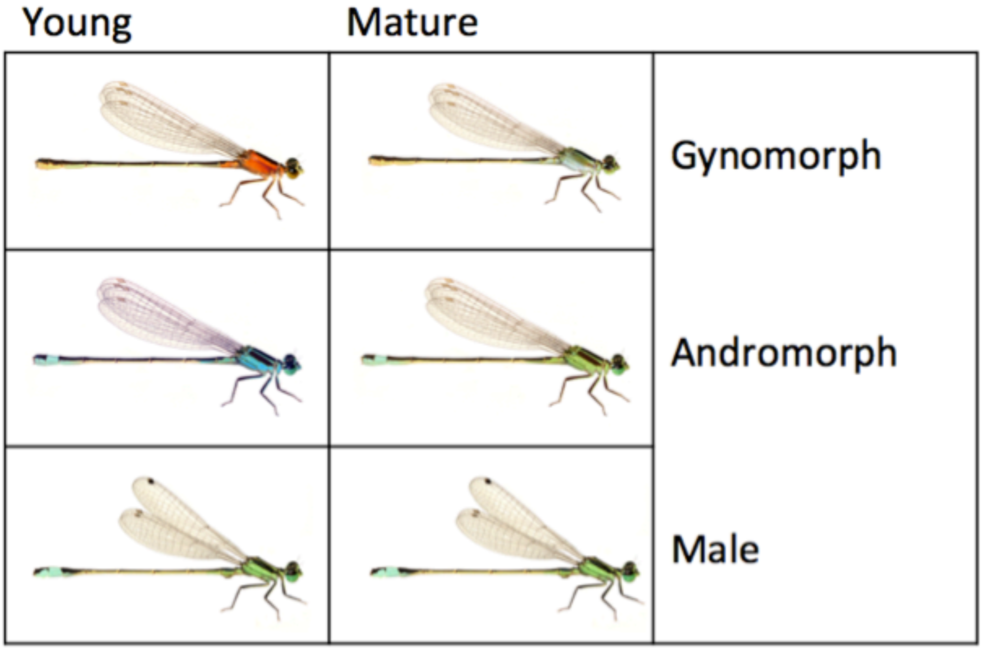

Figure 3.1 Developmental color change in *Ischnura ramburii*. Illustrations from Abbott 2012.

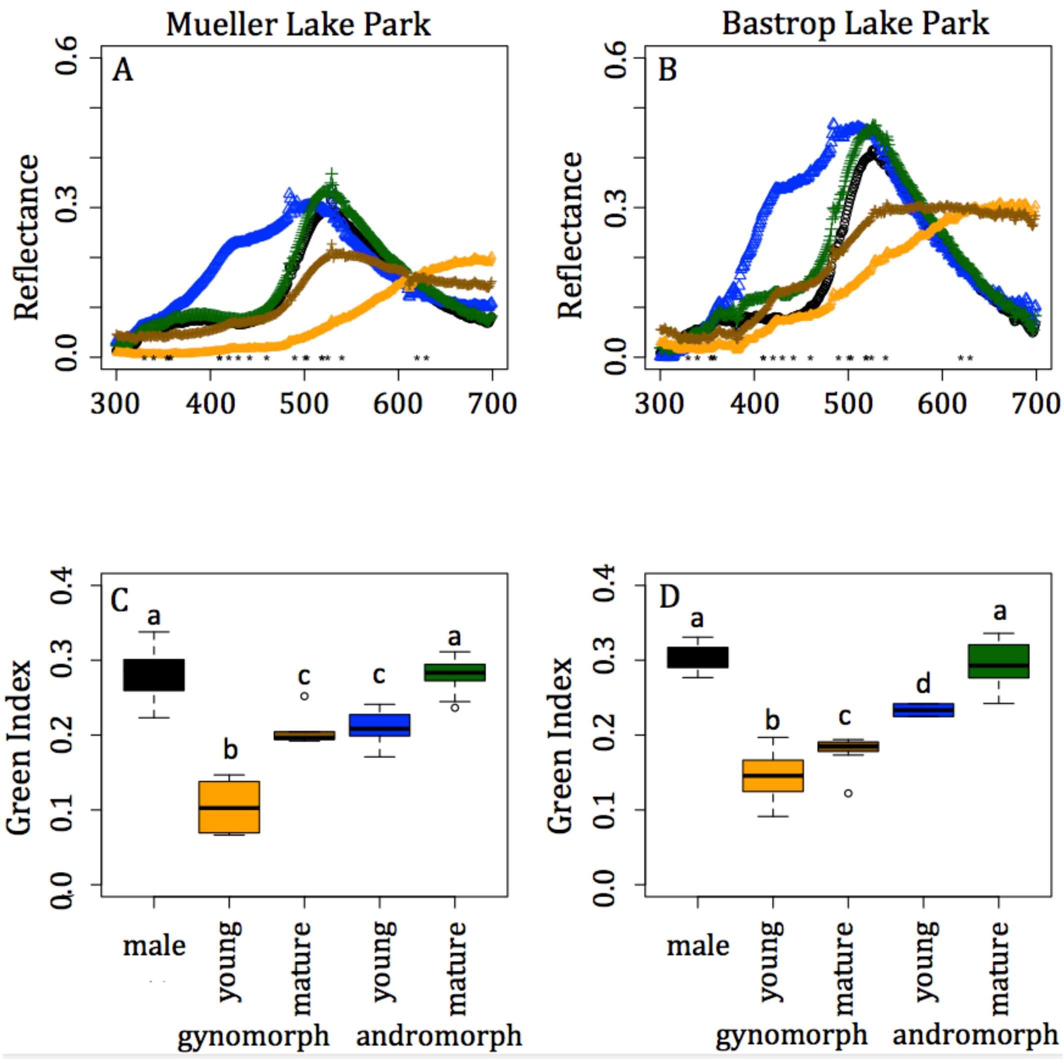

Figure 3.2 Spectrophotometric data from two Texas populations of *Ischnura ramburii*. Males of the two age classes did not differ in spectral properties (see results), and were therefore pooled. AandB: reflectance curves of young and mature gynomorph and andromorph females. $\lambda_{max}$for opsins reported from several odonates (Briscoe and Chitka 2001) are denoted by asterisks. CandD green indexes (500-550nm reflectance /350-700nm reflectance) obtained from spectrograms of *I. ramburii*. Mature andromorphs were statistically indiscernible from males, whereas younger counterparts and gynomorphs were spectrally dimorphic.

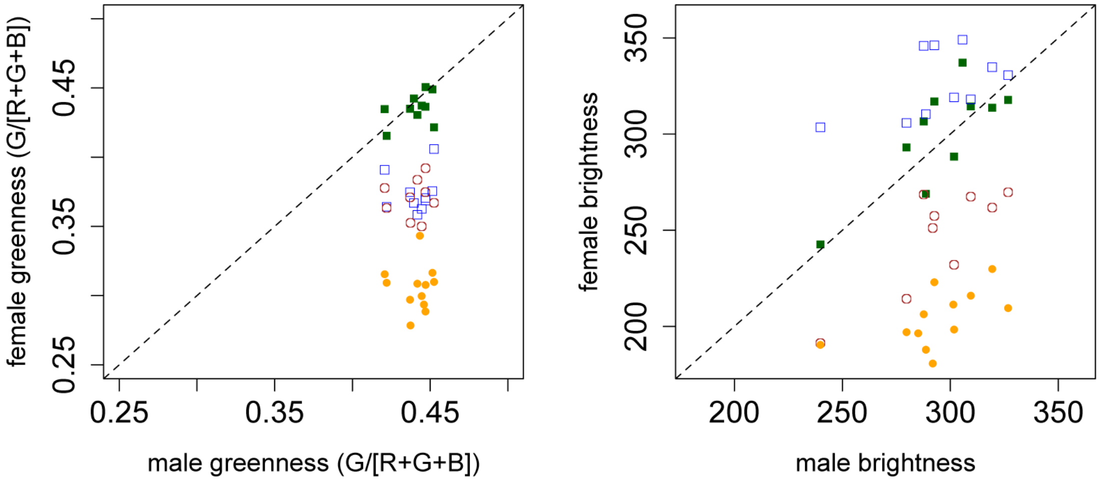

Figure 3.3 Population means for *Ischnura ramburii* female coloration (andromorph n=159, gynomorophs, n=207) relative to that of syntopic males (n=201). Female morphs are coded by color as in Figure 2; andromorphs are represented by squares, and gynomorphs by circles; filled symbols indicate mature individuals whereas hollow ones denote young ones. Dashed lines indicate the 1:1 ratio predicted by the male mimicry hypothesis. Mean brightness was correlated between andromorphs and males ($p<0.02$, adjusted R-squared: 0.53).

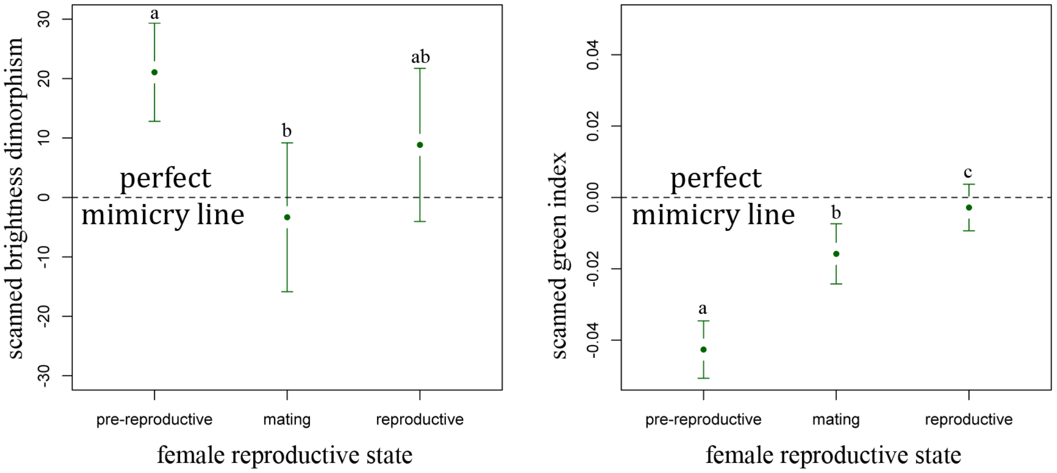

Figure 3.4 95% confidences intervals for differences between *Ischnura ramburii* andromorph and syntopic male color across 10 populations, as a function of andromorph reproductive state. Both mating and reproductive, solitary andromorphs resembled males more closely than virgin (non-mating/pre-reproductive) counterparts in brightness (left panel). Only post-mating, reproductive females met the predictions of perfect mimicry (95%CI bounding zero, indicated by dashed line) for both brightness and green index (right panel).

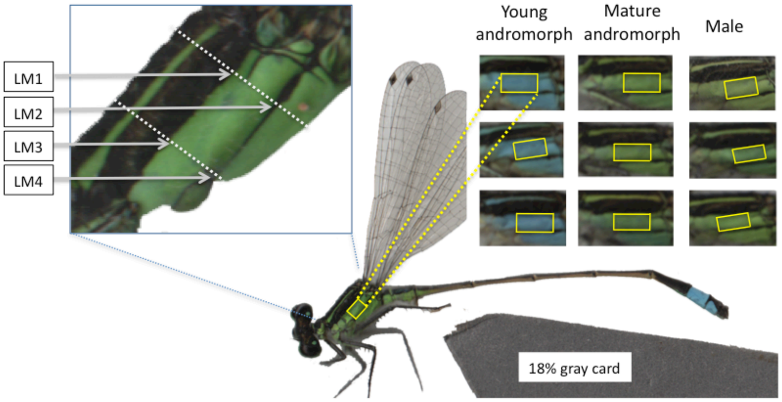

Figure S1. Representatives of scans collected from *I. ramburii* andromorphs and males from both young and mature age classes. The yellow rectangles indicate the region of interest within each scan, across which pixel saturation of R,G, and B color channels were averaged for each individual. This region was defined using the four landmarks shown in the inset: LM4 is the joint between the Metepisternum, Metepimeron, and Posterior Coxa. LM2 is the point along the Metapleural suture that is bisected by a line running perpendicular to the Metepisternum, and immediately anterior to the short melanic line which divides the Mesipimeron and Metepisternum, LM3 and LM1 are found by drawing lines perpendicular to the long axis of the thorax that intersect LM2 and LM4, and selecting points along the margin of the Antehumeral stripe. For definitions of anatomical terms see Abbott (2008).

Figure 3.S1. Scanning methods used for spectral analyses of *Ischnura ramburii*

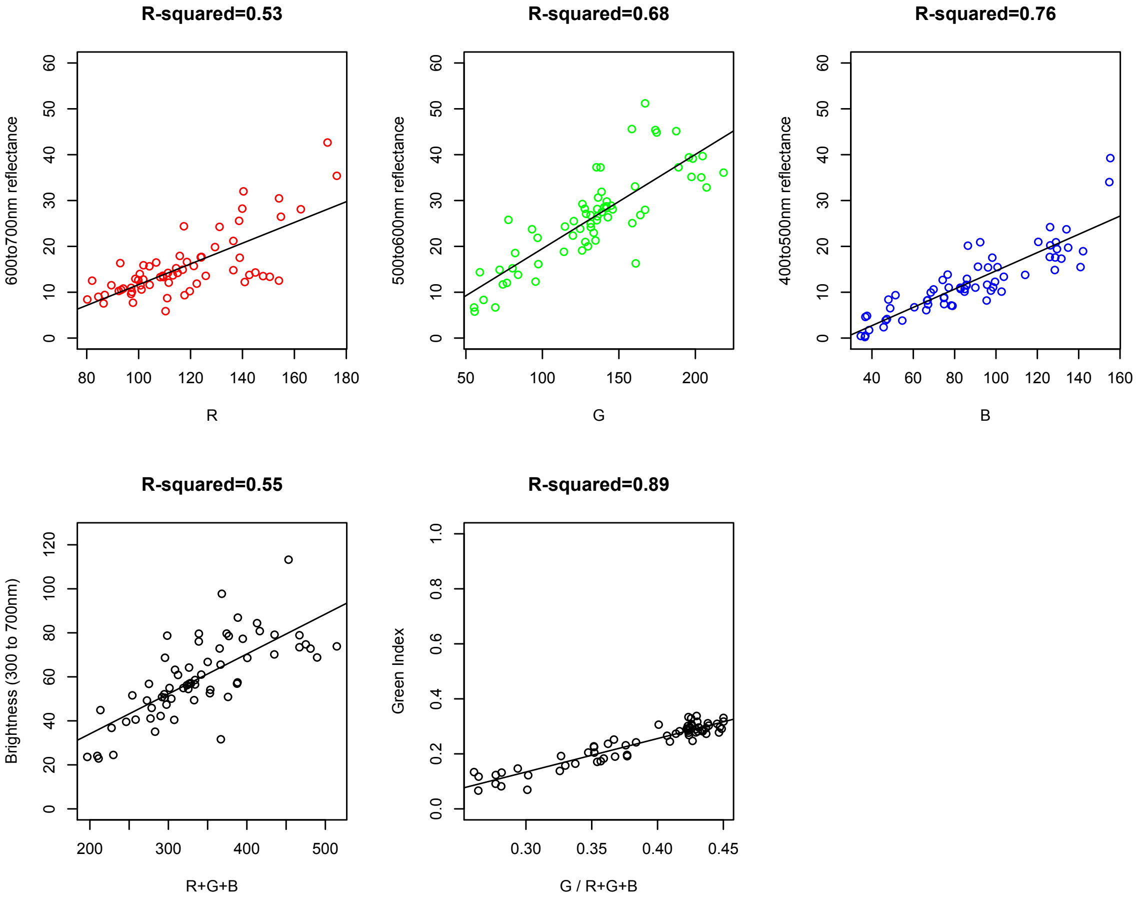

Figure 3.S2. Comparison of spectreal indexes obtained from field caught *Ischnura ramburii* using spectrophotometry (y-axis) and digital scanning (x axis).

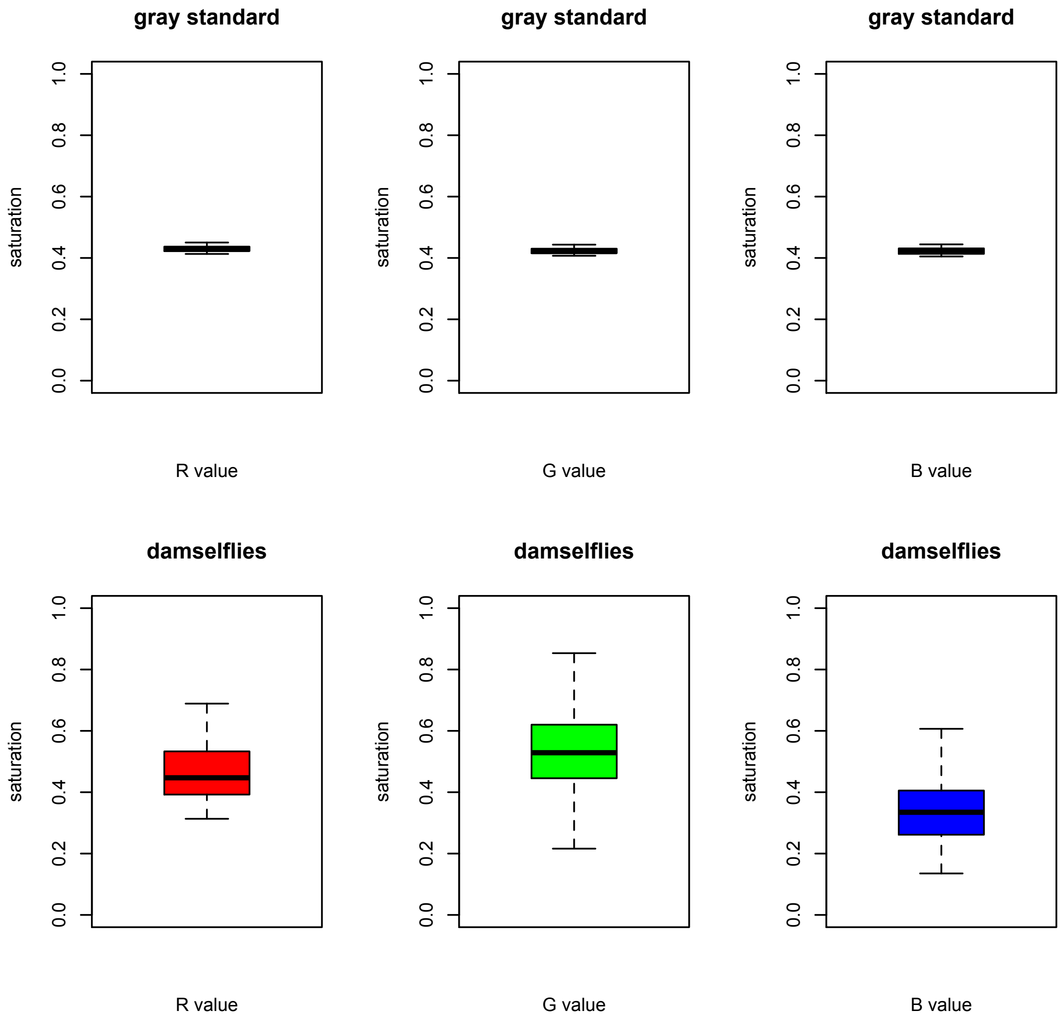

Figure 3.S3. Evaluation of repeatability of RGB exposure across digital scans of *Ischnura ramburii*. Gray standard variation was minimal in comparison with among-individual variation for all three color channels.

Table S3.1: Sampling localities for spectral analyses of *Ischnura ramburii* using spectrophotometry (in parenthesis) and scanner [RGB] data.

Region	Locality (year)	GPS coordinates	Young andromorphs	Mature andromorphs	Young gynomorphs	Mature gynomorphs	Males
Texas	MLP (2011)	30°17'22"N 97°42'5"W	(6) 7	(3) 13	(4) 7	(7) 14	(33) 55
Texas	BLP	30°8'26"N 97°17'6"W	(2) 16	(14) 10	(6) 21	(4) 8	(15) 6
Oahu Island	MPA (2010)	20°12'11"N 155°43'55"W	(1) 8	(9) 3	(2) 5	(3) 5	(19) 6
Kauai Island	WTF (2010)	22°11'53"N 159°28'2"W	(-) 3	(-) 9	(-) 12	(-) 7	(-) 13
Kauai Island	NAW (2010)	21°57'38"N 159°21'10"W	(3) 2	(2) 11	(7) 26	(16) 23	(18) 51
Kauai Island	TAR (2010)	22°12'6.39"N 159°29'52"W	(2) 2	(5) 7	(4) 2	(6) 3	(10) 7
Kauai Island	WCR (2010)	21°57'46"N 159°40'5"W	(-) 4	(4) 6	(7) 3	(5) 9	(8) 28
Hawaii Island	POL (2010)	20°12'11"N 155°43'55"W	(-) 1	(-) 6	(-) 4	(-) 5	(-) 8
Oahu Island	SLP (2011)	21°21'11"N 157°54'34"W	(-) 4	(-) 11	(-) 3	(-) 1	(-) 11
Kauai Island	PRV (2010)	22°13'26"N 157°54'34"W	(-) 7	(-) 16	(-) 7	(-) 11	(-) 13
Total	-	-	(14) 54	(37) 92	(30) 90	(41) 86	(103) 198

Chapter 4: Male-like females increase male-male interactions, and reduce male fitness, in a female-polymorphic damselfly

Time will heal a broken heart, just like time will heal his broken arms and legs.
- Miss Piggy

ABSTRACT

Sexually antagonistic coevolution can drive both population dynamics and speciation, but many potential effects of conflict-related traits have not been explored. This study examines a damselfly, *Ischnura ramburii*, in which two female color morphs co-occur, and examines the role of female morph frequency in shaping male-male interactions and its consequences for male fitness.

In female polymorphic damselflies (including *I. ramburii),* "andromorph" females, which resemble males, coexist with non-male-like "gynomorph" females. Andromorphs are thought to mimic males in order to avoid harassment, but males can also learn to cue on the prevailing morph and thus exert frequency-dependent harassment. I hypothesized that, in the presence of andromorphs, male-male interactions would increase due to increased sex recognition errors. As predicted, mesocosms populated with andromorphs showed more male-male interactions than mesocosms populated with gynomorphs. Further, increased andromorph frequency and male-male interaction was associated with significant reduction in male condition (body mass) and survival. Taken together, these findings suggest that a resistance trait (male-mimicry) can have indirect effects on male fitness components. These effects may be important for understanding the dynamics and consequences of sexually antagonistic coevolution.

INTRODUCTION

Since Darwin proposed his theory of sexual selection, and since Trivers resurrected this theory in the 1970s, conflict between the sexes has been assumed to be an implicit aspect of many mating systems (Darwin 1871, Trivers 1972). This conflict has since been understood to drive coevolutionary arms races and play a role in speciation (e.g. Holland and Rice 1999, Gavrilets 2000, Arnqvist and Rowe 2005). One widespread and well-studied form of reproductive conflict involves differences in the optimal mating rates of males and females: whereas male reproductive success typically increases with each mating event, female reproductive success often plateaus or declines with additional matings after an optimal number is reached (Bateman 1948). Intersexual conflicts over mating rates are important because they can promote the elaboration of male traits that manipulate female reproduction (Holland and Rice 1999) and also the evolution of female tolerance or resistance to unsolicited mating attempts (Svensson and Raberg 2010, and references therein).

Compounded across individuals, fitness costs of sexual conflicts can scale up to influence population-level demography, including (perhaps most importantly) intrinsic growth rate. While a handful of studies have examined the effect of sexual conflict on population dynamics and persistence (e.g. Rankin et al. 2011) the mechanism through which conflict might affect demography is assumed to be straightforward: conflict-related traits could reduce growth rates by imposing costs on their deployers and/or targets, and other individuals in the population are not generally thought to be affected. Yet in principle, conflict-related traits could also have unintended feedbacks on individuals that are not intended as targets. These feedbacks remain virtually unexplored, but might likewise affect both individual fitness and population dynamics.

In the present study, I use female-polymorphic damselflies, an unusual system in which some females are thought to mimic males in order to avoid harassment, to ask if and how a female resistance trait might indirectly feedback on male fitness. Since male search images are shaped by previous female encounters (Miller and Fincke 1999, Van

Gossum et al. 2001, Takahashi et al. 2010), I hypothesized that 1) higher frequencies of male mimics (andromorphs) would cause increased male-male interaction due to mistakes in sex recognition, and 2) costs of increased male-male interaction would culminate in reduced male performance. This would provide an example of an indirect feedback of a conflict-related trait; male-mimicry may not only help andromorphs evade mate-searching males, it might also increase interactions between males due to sex recognition errors. To test this, I manipulated the ratio of male-like females ("andromorphs") to those of an alternative female morph ("gynomorphs") in experimental mesocosms containing the damselfly *Ischnura ramburii* (Rambur's Forktail; Figure 4.1). I then quantified the impact of andromorph frequency on rates of male-male interaction, and assessed the effects of female morph-frequency on male survival and condition. These experiments revealed that under high-density conditions, the presence of andromorphs was associated with increased male-male interactions, which in turn were associated with decreased male condition and survival. Thus, a resistance trait, andromorphy, may have indirect effects on same sex-interactions, and these effects could be important for both population dynamics and polymorphism persistence.

METHODS

Individuals of the species *Ischnura ramburii* were collected in July 2011 and June 2012 from an invasive population inhabiting a stream next to the CTAHR research station in Waimanalo, HI, which has a species-typical andromorph frequency of ca. 0.33

(Gering unpublished). Invasive populations were used because this work was part of a larger research program that seeks to understand post-invasive evolution in this species. Thus far, however, morphology and behavior are largely similar between the native (Texas) and invasive (Hawaiian) populations studied,

After capture, each male was dusted with UV-fluorescent powders (DAYGLO, Cleavland, OH, USA) on his claspers and copulatory organ. Male damselflies clasp their mates behind the head to initiate mating, which is followed by coupling of their copulatory organs to receptive partners' vaginas. Thus, the presence of dust on a female's dorsal thorax indicates copulation attempts (Gosden and Svensson 2009). While male damselflies deploy a suite of pursuit and display behaviors during male-male interactions (lunging biting, etc.), male-male claspings (which are common in scramble mating species such as *I. ramburii*) are typically interpreted as sex recognition errors (e.g. Robertson 1985, Van Gossum et al. 2001). Claspings can also be unambiguously diagnosed by the presence of dust on the dorsal thorax.

Dusted males and unmarked females were released into 10-foot by 8-foot mesh enclosures at the CTAHR field station, which were outfitted with a shallow cement-mixing tub filled with water; enclosures did not have floors, allowing natural vegetation to serve as perches and shelter. Experimental populations were comprised of 8 (low density) or 16 (high density) individuals, with a ratio of 5 males to 3 females across experimental replicates, and one of four andromorph frequencies (ranging from 0% to 100%; Figure 2). During the experiments, *Drosophila melanogaster* were introduced in proportion to damselfly density (1 vial/2 damselflies); damselflies were observed foraging and feeding in all experimental trials. After two days, surviving *I. ramburii* individuals were removed from the tents, examined for dust, and placed in acetate envelopes for weighing on a portable balance (HandC model S123).

To examine how male-male chases, which do not always culminate in clasps, were affected by morph types, in 2012 I also conducted focal observations on 60 males under identical conditions but restricting morph frequency to either 100% gynomorph females (n=3 high density replicates) or 100% andromorph females (n=3 high density replicates). For the observational replicates, each male was marked with a unique number on the wing, and followed for one or two ten-minute observations commencing in the afternoon (with the second observation dependent upon overnight survival). Results from the two focal periods were averaged.

Focal observations were initiated beginning 2 hours after populating mesocosms. Before and during observations, I sat on a stool in the corner of the enclosure, allowing 20 minutes for damselflies to acclimate to my presence. I then selected a focal male, and recorded for 10 minutes the number of times he was approached by other males, approached other males, and/or approached a female. I then proceeded to observe other males within the tent until all had been observed. Since these experiments were intended to quantify pre-clasping interactions only, males were not dusted. Observations were excluded in which it was not possible to determine which individual initiated an interaction. Nine individuals were not resighted during focal observations, thus a total of 25 males were observed from andromorph-only treatments, and 26 males were observed from gynomorph-only treatments.

All results were analyzed using mixed models, with experimental replicate as a random effect using the lme4 library in the open source software package R (R Core

Development Team 2009). For non-observational replicates, the predictor variables were morph frequency, density and the density*morph frequency interaction. Andromorph frequency was fitted with restricted cubic splines using the Design library, but adding knots did not improve fitted models using AIC criteria. Response variables examined were survival and clasping (binomial; measured as Y/N) and mass (Gaussian; measured in mg). For the observational data, the response variables examined were approaches received from males, approaches directed at females of each morph, and approaches directed at males (Poisson).

RESULTS AND DISCUSSION

Male mortality is density and frequency-dependent

I predicted male-male interactions would increase in the presence of male mimics, and negatively affect male fitness. These predictions were upheld by observed mortality (n=64 of 215 individuals), which increased in the presence of andromorphs (p=0.025), as well as in the higher density treatment (p=0.004; Figure 4.2). The interactive effect of frequency*density on mortality approached, but did not reach significance (p=0.099), with a trend towards greater effects of andromorph frequency on male mortality at high (vs. low) density (Figure 4.2). An interaction between frequency and density could be expected if male-andromorph encounters are more common within dense populations, and thus promote learned responses to male-like targets, and/or due to elevated rates of male-male encounter within dense and andromorph-rich populations (e.g. increased intrasexual competition). In either scenario, the lack of significance in the interaction term could reflect insufficient power and/or a lack of more extreme density treatments in the experimental design.

Most males that died were never recovered. Though tents were left unused for three days between experiments, and searched daily for any missed individuals, only two were found, and these were excluded from analyses. Observed causes of mortality included spider predation (inferred from bodies found in webs; n=7 males), cannibalism by females (inferred from direct observation; n=3 males eaten by two gynomorphs and one andromorph), and other/unknown causes (inferred from recovery of unharmed bodies, potentially indicating exhaustion or overheating; n=4 males). Although experiments were conducted in as natural a setting as possible, the type and intensity of mortality was almost certainly affected by the mesocosm environment. Nevertheless, energetic and/or vigilance costs of male-male interactions are likely to be present and important for male fitness in both mesocosm and field settings. An increase in male-male interaction brought about by increased andromorph frequency would thus likely affect males similarly in both contexts.

Male-male interactions are associated with decreased male condition

Patterns of male condition (body mass) across treatments resembled those observed for mortality: body condition was lower for males removed from high-density replicates than for males removed from low density ones (p=0.004) and negatively related to andromorph frequencies in high density replicates (p=0.028; Figure 4.2).

It appears that these effects cannot be explained by increased rates of male-male clasping: dust on the thorax (indicative of male clasping) was observed on only 18 of 165 surviving males, and the proportion of males clasped did not differ between high and low density replicates (6 of 64 low density survivors, 12 of 82 high density survivors; Fisher's exact p=0.45). Only one of the 14 male bodies recovered had dust on his thorax, again suggesting male-male clasping was not directly responsible for mortality. Focal observations, described below, suggest instead that pre-contact harassment may underlie observed survival declines.

Male-male interactions increase with andromorph frequency

Focal males lunged at other males more frequently when housed with andromorph females (Figure 3, z=-2.44; p<0.015). Focal males also trended towards receiving more lunges from other males when housed with andromorphs, though this trend did not reach significance (z=-1.64; p=0.102). In contrast to these patterns, male-female interactions did not differ between treatments (z=1.42; p=0.157), and, notably, were rarer than male-male interactions by an order of magnitude (Figure 4.3).

CONCLUSIONS

Taken together, these results indicate that male-male interactions can reduce male fitness (body mass and survival), and are exacerbated by the presence of male-like females (andromorphs). This could result from males mistaking other males for prospective mates, as a consequence of previous encounters with male-like females. Under this scenario, energy and/or vigilance expended on the pursuit and avoidance of other males could culminate in the condition declines and mortality I observed in experimental mesocosms. Alternatively, since andromorphs are more resistant to male clasping than gynomorphs (Gosden and Svensson 2009, Gering unpublished), and also mate fewer times, it is possible that males simply exert more energy pursuing them, and must deploy broader search images. This seems unlikely to explain experimental outcomes, since focal males in the observational study approached andromorphs and gynomorphs with equal frequency (Figure 4.3). Importantly, however, both small sample sizes and the absence of data on female receptivity preclude robust testing of this hypothesis.

There has been much interest in identifying the mechanisms that maintain female-polymorphisms, which occur within >100 species of damselflies (Fincke et al. 2005). The reproductive success of female morphs has been shown to be both density and negative-frequency dependent, and these factors are sufficient to maintain polymorphism (Svensson et al. 2005, Takahashi et al. 2010). The present study indicates, however, that female morph frequencies could also influence male fitness, thus altering both the social environment in which female morphs compete, and the dynamics of populations in which they co-occur. It is intriguing, for example, that a purported male mimic, who would have an advantage over gynomorphs in high-harassment environments, might also attenuate harassment by decreasing male survival or condition, undoing its advantage via indirect and deleterious effects on male fitness.

The complex interplay between sexual conflict and male fitness variation reported here need not be limited to polymorphic damselflies. In bedbugs, for example, male traumatic insemination of other males has imposed sufficiently strong selection to promote evolution of male counter-defense (Ryne 2009). Because males are often regarded to have the upper hand in sexually antagonistic coevolution, few investigators have quantified the deleterious effects of female resistance on male fitness. The present study highlights how sexual conflict can have important and unexpected feedbacks on male fitness, which merit further study to gain a richer understanding of how sexual conflict can promote biodiversity.

Figure 4.1 Mating pairs of *Ischnura ramburii*. The andromorph female (bottom right) resembles the male (top left and right) in both coloration and behavior, whereas the gynomorph (bottom left) does not. Photos provided by Steven Coleman (left) and Ken Slade (right).

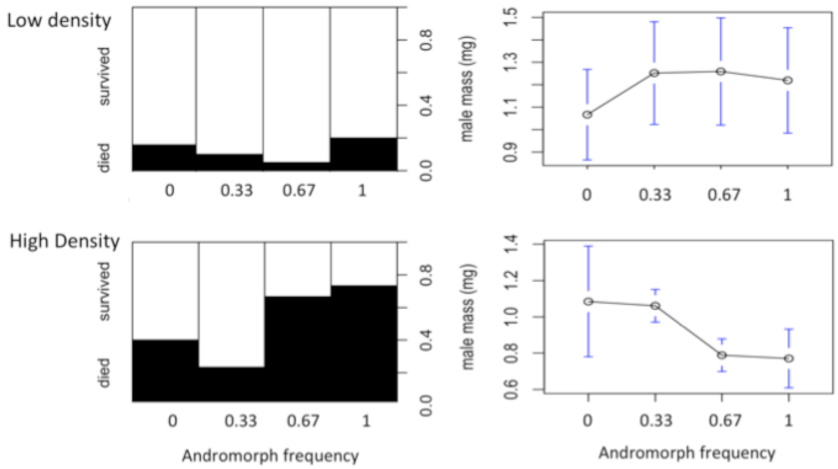

Figure 4.2 Mortality (left panels) and condition (right panel) of male *Ischnura ramburii* in experimental mesocosms. At high population densities (top panels), males that were housed with andromorphs had increased mortality rates, and survivors had lower body mass at the experiment's conclusion.

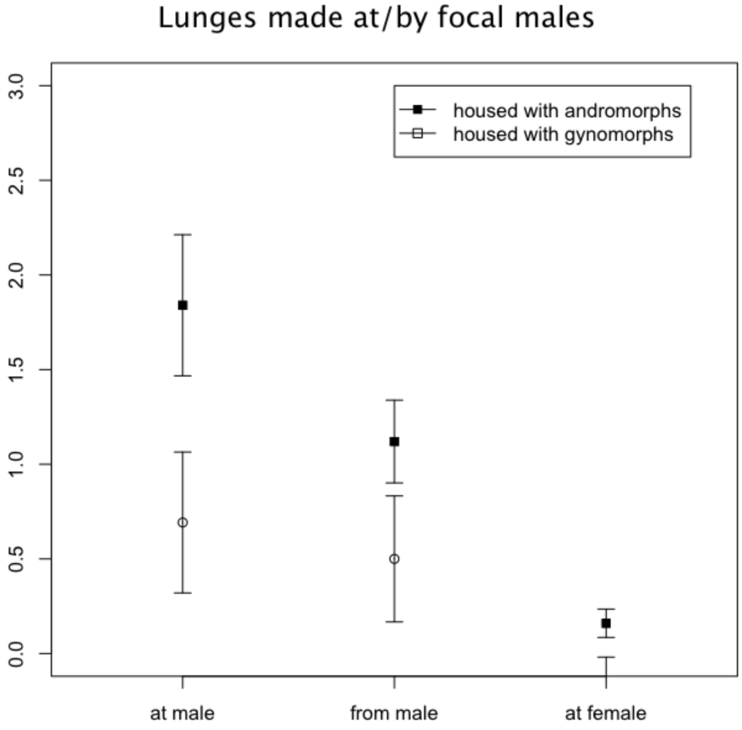

Figure 4.3 Lunges observed during 10-minute focal observations of males that were housed in experimental mesocosms at high density (16 individuals/tent) with either andromorph females (filled circles, n=25 males) or gynomorph females (hollow circles, n=26 males).

Chapter 5: Rapid evolution of andromorphy in an invasive damselfly is attributable to density-dependent and frequency-dependent mating conflict

-Eben J Gering

ABSTRACT

This study documents rapid evolution of female polychromatism following the introduction of a damselfly (*Ischnura ramburii)* into Hawaii. Two female color morphs (andromorphs, gynomorphs) occur throughout this species' native range, but all surveyed Hawaii populations were female-monomorphic (gynomorph only) as recently as 1990. Sampling between 2008-2012 revealed that at least two islands now appear to maintain stable female-limited polymorphism. On Oahu, a relationship exists between field morph frequencies and densities among sampling localitities, indiciating that frequency-dependence and density-dependence may influence andromroph and gynomorph fitness to promote polymorphism. This hypothesis was tested by manipulating frequencies of andromorphs and gynomorphs in experimental mesocosms at high and low density-regimes. Mesocosm experiments on Oahu indicated that female morph frequency and population density interactively influence female fitness proxies. In low-density treatments neither morph was subject to frequency-dependence in measured fitness components; however, gynomorphs laid more eggs. In the high-density treatments, only gynomorphs suffered from negative-frequency dependence in survival and harassment. These findings may explain why andromorphs were positively selected after invading populations reached high densities. Density- and frequency-dependence in male-female

interaction rates and female fitness components could also explain how polymorphism is stabilized by feedback between demography and female morph performances within established populations. Density-dependent mating conflicts can be illuminated by, but are not reliant on, sex-limited polymorphisms. Thus the conflicts elucidated here are likely important selective forces in other invasive, sexually-reproducing taxa. This study demonstrates how intersexual conflict can modulate frequency-dependence, generating complex feedbacks that can enrich phenotypic diversity.

INTRODUCTION

The ubiquity and extremity of sexual dimorphism found in nature are often cited as evidence of sexual selection's diversifying powers (e.g. Andersson and Simmons 2006), yet very few studies have documented rapid sexual trait evolution in the wild (Svensson and Gosden 2007). As a consequence, it remains unclear whether mechanisms of sexual selection emerging from theoretical, laboratory and comparative studies can predict evolutionary dynamics that occur over short time scales in nature. One notable instance in which empirical work is lagging concerns the role of mating conflicts in male and female trait evolution (Anrqvist and Rowe 2005). Lab studies suggest mating conflicts can result in rapid evolution (e.g. Rice 1998) and theory indicates that these can in turn influence both population persistance (e.g. Kokko and Rankin 2006) and speciation (Gavrilets 2004). Studies of contemporary evolution within natural populations, where possible, can show how sexual conflict drives evolution in the presence of other selective and stochastic forces.

One of the foremost challenges in documenting conflict-driven evolution is identifying contexts in which it is likely to be observable. I propose that biotic invasions provide unique opportunities to study sexual conflict, because invading populations often experience reduced competition/predation and are found at higher densities compared to their non-invasive counterparts. Enemy escape could increase sexual conflict's primacy over other selective agencies; increased density, by escalating male-female interactions, could increase their *absolute* impacts.

While invasions can provide unique opportunities to study causes and outcomes of mating conflict, there are few tractable in which females exhibit easily discernable and heritable phenotypes that are known or suspected to influence mating interactions. Damselfly color polymorphisms provide an ideal system to investigate sexual conflict. In >100 damselfly species, there occur two or more distinctive female color morphs. "Andromorph" females resemble males in coloration, whereas "gynomorph" (or "heteromorph") females do not. This color variation follows Mendellian inheritance with sex-limited expression in several *Ischnura* species (reviewed in Van Gossum 2008, Takahashi and Watanabe 2004, Gering, Huckabee and Locklin unpublished data), and is thought to be maintained by frequency-dependent sexual harassment. Andromorphs may effectively mimic males to evade detection (Robertson 1985) and/or males may harass the prevailing morph due to learned mate recognition (Fincke 2004). Field studies have shown that the performance of alternative female morphs correlates with population density (e.g. Van Gossum et al.2008), and morph frequency (e.g. Svensson et al. 2005, Takahashi and Watanabe 2010), and is affected by male-female interactions (e.g. Gosden and Svensson 2009, Gosden et al.2011). Few studies, however, have experimentallymanipulated demographic parameters and these that have yielded mixed results concerning the fitness of alternative color morphs (e.g. Xu and Fincke 2010, Sirot and Brockmann 2001). Importantly, no study has manipulated population density under semi-natural conditions. Density may have important effects on the frequency-dependence of mating conflicts because densities can shape male-female interaction rates. Finally, studies have not related experimental effects of demographic manipulations to observed spatiotemporal variation occurring in natural populations.

The present study capitalizes on the invasion of a female-polymorphic damselfly, *Ischnura ramburii* into the Hawaiian archipelago in the 1970s (Harwood 1976). Whereas andromorphs were at ca 1% frequency less than 30 years ago (Hilton 1989), field surveys over a period of four years reveal andromorphs have since reached high frequency on multiple islands. The goals of this study were 1) to assess the evidence for recent selection on andromorphy within invasive populations, 2) to characterize the relationship between andromorph-frequency and population densities among contemporary populations, 3) to test whether andromorph and gynomorph performance are frequency-dependent, and 4) to test for frequency-dependence under low and high-density conditions representing the initial and later stages of biotic invasion.

METHODS

Obtaining morph-frequency data for the native range

Morph frequencies from the native range of *I. ramburii* were obtained from published studies, from field sampling of natural populations throughout Central Texas, and from museum collections visited opportunistically (Table S1). One assumption of using museum specimens to obtain morph frequencies is that all accessioned material was contributed by collectors who were unbiased and equally effective at catching each morph. This assumption was supported by: 1) the lack of female-monomorphic samples from any well-sampled locality, indicating that collectors were aware of morph coexistence 2) similarities between field-derived, published, and museum-based morph frequencies (Robertson 1985, Sirot et al.2003) and 3) similar rates of recapture in field studies of marked females, by myself and several field assistants (Gering unpublished).

Field Sampling of invasive populations

A collection of ca 70 streams, lakes, ponds and entrainments on three islands (Kauai, Oahu and Hawaii) were identified using Google earth; no features above 300m elevation were found to contain *I. ramburii,* whereas most perennial water features at low elevation harbored the species. A subset of these localities were selected based upon accessibility and to maximize geographic spread and habitat diversity for this study.

Each site was visited between 10am and 4pm, which spans the observed period of peak activity for the species (see also Robertson 1985). Sites were revisited on multiple days to reduce impacts of daily climatic and/or demographic fluctuations. Most sites were also visited in multiple years. During each visit, field assistants walked along and through shoreline vegetation and collected *I. ramburii* using aerial nets (Bioquip). All captured individuals were kept in acetate envelopes until a given survey's conclusion, and individuals' sex, morph type and mating status (mating or not mating) were noted upon capture. Population densities were determined by dividing the number of captured individuals by the collective amount of time invested in surveys. Because assistants varied in their capture abilities, and often improved over any given season, their search time was always weighted. I multiplied their search time by (their total captures / my total captures). Results did not differ qualitatively from those obtained by restricting density estimates to (captures/search time) for myself alone.

Testing for recent selection on andromorphy

A neutral hypothesis for morph frequency change within Hawaii was tested using forward simulations, and a diffusion approximation of genetic drift (Kimura 1980, as in Kay 2007) iterated across a range of population sizes (100,000 replicates per effective population size, N_e) and parameterized with generation times from a lab-rearing experiment with Texas *I. ramburii* (Gering, Huckabee, and Locklin unpublished data). Rearing times were similar to published development times for multiple congeners (Johnson 1966) and a small sample of Hawaiian *I. ramburii* (Gering Chapter 2). To maximize the opportunity for neutral processes to drive morph-frequency change, I assumed year-round breeding in Hawaii (i.e. maximal number of generations elapsed between historic and contemporary surveys). Phenotypic frequencies were taken from published data (Hilton 1989), and monomorphic populations were assumed to have an andromorph frequency of (1/ (Nsampled +1)). This provides a liberal estimate of drift's power to increase morph frequencies by assuming andromorphs to have been present at the maximum possible frequencies, given the historic data.

Density and frequency-manipulations within experimental mesocosms

Experiments were carried out at a low elevation field site on Oahu Island (the University of Hawaii's Center for Tropical Agricultural Research - CTAR) adjacent to a dense population of *I. ramburii*. Field-caught damselflies from the population at CTAR were aged based on thorax color and wing-wear (Corbet 1999). Young adults were randomly assigned to experimental populations, which were housed in screen-walled tents (10feet x 10feet x 8feet) enclosing streamside vegetation and equipped with plastic tubs containing water and grass to allow drinking and maintain humidity. Each morning, populations were fed with one small culture vial of *Drosophila melanogaster* per each damselfly in a tent. Casual observations indicated that within experimental enclosures, test subjects demonstrated previously described and species-typical behaviors for the duration of the experiments (feeding, drinking perching, chasing, mating, grooming; Robertson 1985, Sirot and Brockman 2001).

To determine the frequency and outcome of male mating attempts, I modified a newly developed protocol that takes advantage of the damselfly's unusual reproductive biology (Gosden and Svensson 2007, Xu and Fincke 2011). Males use specialized abdominal appendages to clasp females posterior to the head, but females control copulation initiation by bringing their abdomens into contact with male thoracic genitalia (Fincke 1997). Each male's claspers and genitalia were dusted with one of six UV-fluorescent powders (DAYGLO, Cleavland, OH, USA), which are transferred to females by physical contact. At the end of experiments, individuals were examined by microscope under UV illumination. Colors present on female thoraxes and abdomens then provide conservative, minimum estimates of both male claspings and female propensity to accept matings. My control studies showed that dust is successfully transferred by contact

(14/14 clasps, 11/11 matings), and false positives do not occur when females, but not males, are dusted (0/20 focal ♀♀). For additional tests of this method see Gosden and Svensson (2008) and Xu and Fincke (2012). Two pairs of fluorescent powder colors (red/orange and green/yellow) proved difficult to distinguish during data collection, and observations of these colors were therefore pooled to give a total of 4 unique colors/tent.

Experimental populations were comprised of 8 (low density) or 16 (high density) individuals, with a ratio of 5 males to 3 females across experimental replicates, and one of five andromorph frequencies (ranging from 0% to 100%). The densities these treatements established within tents were selected to approximate the lower and upper boundaries observed at shorelines during 4 years of field sampling on Oahu. This range of densities is similar to that observed from other localities (see Figure 5.3). Each female was uniquely numbered, to ensure missing individuals were not carried across replicates. After 24 hours, all individuals were removed from tents using aerial nets, following extensive searching by multiple observers. Tents were searched again before beginning new replicates, and only two individuals (both male) were discovered after being missed during previous searches.

Surviving females removed from tents were given an additional 48 hours to lay eggs in oviposition chambers, with clutch sizes providing a cross-sectional fitness index (Svensson et al.2005).

Statistical analyses

All analyses were conducted using the open-source software package R (R Core Development Team 2010). Associations between candidate predictors (female morph,

morph-frequency, density, and interactions) were tested using generalized linear models for the response variables of survival (binomial), clasping (Poisson), and mating (Poisson). Field data for morph-frequencies (binomial) were fit using populations nested within islands as random effects, and density as a fixed effect.

RESULTS

Andromorphy is positively selected within Hawaii, and correlates with density

I. ramburii is polymorphic on both Kauai and Oahu Islands, despite well-documented monomorphism as recently as 1989 (Hilton 1989), indicating extremely rapid evolution of andromorphy between 1990-2008 (Figure 5.1). In contrast, female morph frequencies did not fluctuate dramatically between 2008-2012 at resampled localities on these islands.

Samples from the Big Island (Hawaii) were largely free from andromorphs in 2008-2010 surveys, and therefor excluded from regressions of morph frequency on demography. However, two andromorphs were detected at extremely low frequency in the best-sampled locality, and andromorphs may have gone undetected at other, less well-sampled sites.

I ramburii was found to be polymorphic throughout its extensive native range (Tables 5.S1, 5.S2), with andromorph frequencies of <50%. Data spanning >50 years' time revealed a narrow range of morph frequencies, in stark contrast to findings from Hawaii.

Simulations indicated that genetic drift alone cannot explain observed morph increases on Oahu and Kauai, even in the hypothetical case of very small N_e (e.g. N_e>180). Effective population sizes are difficult to ascertain, but are likely to be much larger than 180. My own fieldwork inidicated that >100 individuals can be caught at most localities on a single day, and survey data suggest that invaded localities have sustained large populations since the early stages of invasion (Harwood 1976, Robertson 1986, Hilton 1989, Polhemus and Asquith 1996, Parham et al. 2008). Simulation results thus corroborate my conclusion that androchromatism likely underwent positive selection within the recent past.

Mixed model analyses revealed a significant correlation between contemporary morph frequencies and population densities on Oahu ($p=0.04$; Figure 5.3). This relationship was not significant for Texas or Kauai.

Experimental evidence for differential density effects on morph fitness

Mesocosm experiments confirmed that density and color morph frequency affect both female fitness components and male-female interactions (Figures 5.4, 5.5). Clasping rates increased at high density, and were higher for gynomorphs than andromorphs (irrespective of morph frequency; $p<0.05$, Figure 5.5). At low densities, rates of male clasping were reduced overall, and did not differ significantly between morphs. Survival rates also exhibited a density-dependent pattern; gynomorph survival was significantly reduced at high density, but only when gynomorphs outnumbered andromorphs (Figure 5.4). Fecundity decreased at high density for both morphs, but was always greater for gynomorphs than andromorphs (Figure 5.S2).

DISCUSSION

Frequency-dependent mating conflicts are density-dependent, and can drive polymorphism in newly colonized habitats

Andromorph frequencies increased dramatically on Oahu and Kauai between 1990 and the present (Figure 5.1). Simulation results (Figure 5.S1) suggested there was selection for andromorphy between 1990-2008, but subsequent field surveys found comparatively stable andromorph frequencies, which mirrored those found in the native range (Figures 5.1, 5.2). Among the Oahu surveys, andromorphs were most frequent in dense populations (Figure 5.3), perhaps indicating dynamic feedbacks between density-dependent mating conflicts and population dynamics (Kokko and Rankin 2006). This possibility was tested with experimental manipulations of density and female morph frequency at a single Oahu locality. Trials revealed that gynomorphs pay a high mortality cost as their frequencies increase under high-density conditions (Figure 5.4), whereas andromporh survivorship revealed no frequency-dependence at either density treatment. These results suggest a survival advantage for andromorphs in high-density environments, which may be due to decreased male harassment.

These patterns can explain andromorph resurgence during later (high density) stages of invasion, but what prevents andromorph fixation? Perhaps when andromorph frequencies increase, population densities decline (owing to andromorphs' lower relative fecundity (Figure 5.S2) and/or indirect, negative feedbacks on male fitness outlined in Chapter 4. Other scenarios can be imagined, but this type of oscillation in frequency and density could, in principle, explain polymorphism persistence between 2008-2012, and the general absence of andromorph-only populations from Hawaii and elsewhere (Table 5.S2).

The invasion context provides a unique evolutionary experiment in which to determine how mating conflicts shape post-invasive evolution. If the same processes operate in both native and invasive contexts, it is curious that morph frequencies appear to converge on the same range (Figure 5.2); this would indicate that male-female interactions could be strong enough to wholly overpower tremendous environmental variation that may exert spatially varying selection on color. Further work is needed to determine the reason *I. ramburii* populations converge on a narrow range of morph frequencies.

Mesocosm experiments provide one of many approaches to identifying determinates of fitness differences between morphs. The levels and types of effects observable within mesocosms are likely influenced by experimental conditions, but are nonetheless insightful. For instance, seminal mesocosm research with *I. ramburii* that varied sex ratios only (i.e. not density) found no advantage for andromorphs at male-biased sex ratios (Sirot and Brockman 2001). In the current study, advantages for andromorphs are only observed under high-density conditions (Figure 5.3). The density and frequency-dependence reported here join a well-rounded body of work suggesting mating conflicts help maintain female polymorphism. Provided the effects (e.g. mortality) recorded in mesocosms stem from mechanisms (e.g. harassment) that are present in nature, they provide insight into how demography can affect fitness components. Experimental outcomes can thus confirm a causal nature for correlations previously observed in the field. In the present case, for example, field and insectary-based studies of *I. elegans* (e.g. Svensson et al. 2005, Van Gossum et al. 2005) previously suggested that density fluctuations and fecundity variation can each stabilize polymorphism, but these have not been tested in manipulative experiments under semi-natural conditions. The current study of *I. ramburii* demonstrates explicitly that dense andromorph frequencies (Figure 5.3) can increase via negative frequency-dependence in gynomorph fitness at high densities. It remains to be seen whether the same mechanisms operate in other *Ischnura* species that have not been subjected to density manipulation. Unfortunately it may prove challenging to find similar opportunities to link experimental outcomes to both long-term morph-frequency dynamics and contemporary distributions.

Our results lend support to the male mimicry hypothesis, since andromorphs benefited from reduced harassment at low frequency, but exhibited potential tradeoffs in fecundity and survival. In both *I. ramburii* and *I. elgans*, andromorphs likewise have lower mating frequencies than gynomorphs in the field (Chapter 2). This is not the case for *I. senegalensis*, in which mature females mate daily, and simple frequency-dependence in male harassment of egg laying females maintains polymorphism (Takahashi and Watanabe 2010). A key difference between this species and *I. ramburii* is that *I. ramburii* andromorphs are chromatically similar to males (Chapter 3), whereas *I. senegalensis* andromorphs are noticeably different to a casual observer. Andromorphs might mimic males more effectively, or exclusively, in taxa where the andromorph-male resemblance is maximal, a possibility that merits further study. It is important to note, however, that the significance of variation in andromorph-male resemblance across species will also depend upon male visual systems and the utility of other sensory modalities for sex recognition.

In summary, density-dependent mating conflicts appear to be important following invasion into new habitats, and can interact with frequency-dependence to both promote and maintain female variation. It is striking that invasion contexts now provide two striking instances of contemporary sexual character evolution and both appear to result from density and frequency-dependence. The invasion of *Ormia ochracea,* a phonotactic parasite, into Kauai Island promoted rapid evolution of a native host, the field cricket *Teleogryllus oceanicus*. Eavesdropping parasites appear to have promoted the evolution of a mutation called “flatwing”, which causes a morphological change that silences male calls. Mute males experience relief from parasitism, but require calling males to occur at some minimal frequency in order to gain access to females, thus the mutation appears, like androchromatism, to have been strongly favored in the past, but persist as a balanced polymorphism.

I. ramburii shows that females can undergo dramatic evolution following invasion into new habitats, and that male-female interactions alone may be sufficient to drive rapid evolution. This species thus provides validation of a well-developed literature on conflict’s potential to diversify traits, and shows that, while some enemies might be left behind during habitat colonization, there is ultimately no escape from the battle of the sexes.

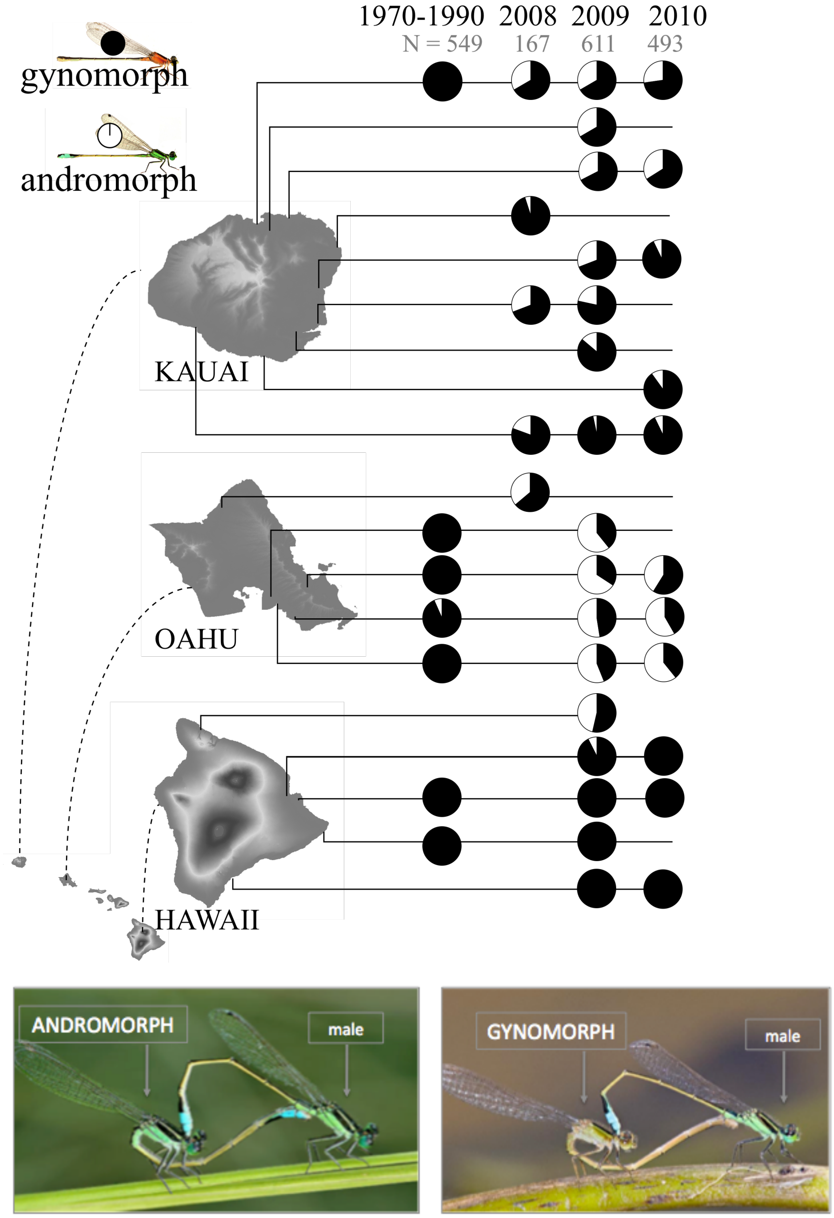

Figure 5.1 Historic (Hilton 1989) and contemporary (Table 1) morph frequencies from 19 well-sampled localities in the introduced range of *Ischnura ramburii*. Frequencies of andromorph females increased on Oahu and Kauai Islands between 1990 and 2008, and simulation results (see Supplemental Figure 1) suggest this resulted from positive selection; Illustrations of andromorph and gynomorph by Barrett Klein as prepared for Abbott (2012), photos by Eben Gering (left) and Tripp Davenport (right).

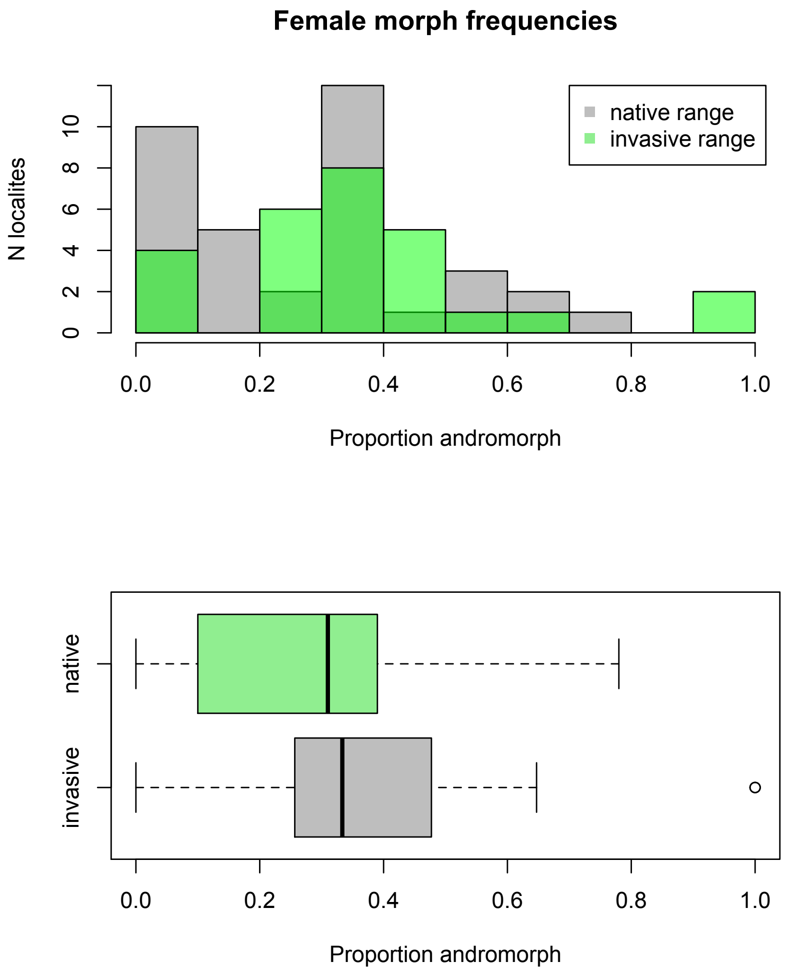

Figure 5.2 Histograms and box-and-whisker plots of andromorph frequencies from the native and invasive range of *Ischnura ramburii* obtained from museum collections and field surveys.

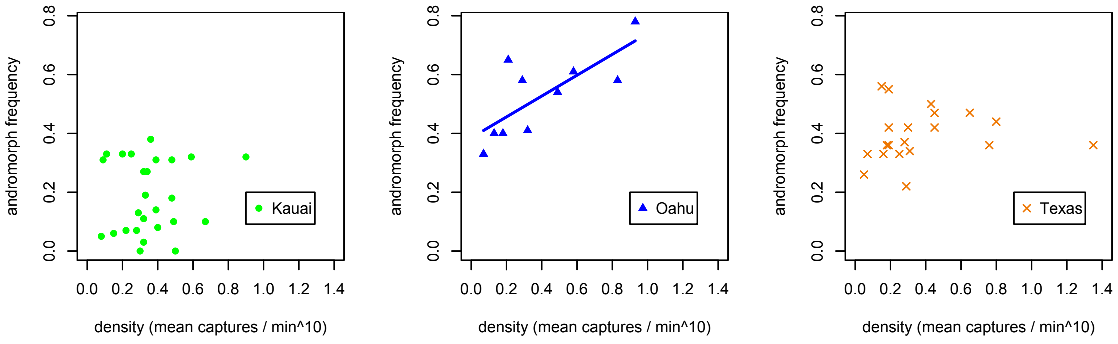

Figure 5.3 A significant and positive relationship was detected in mixed model logistic regression of *Ischnura ramburii* female morph frequencies, with population density (inferred from rates of capture) as a predictor. Random effects of region did not differ between native (Texas) and invasive (Hawaiian) populations sampled between 2008-2011.

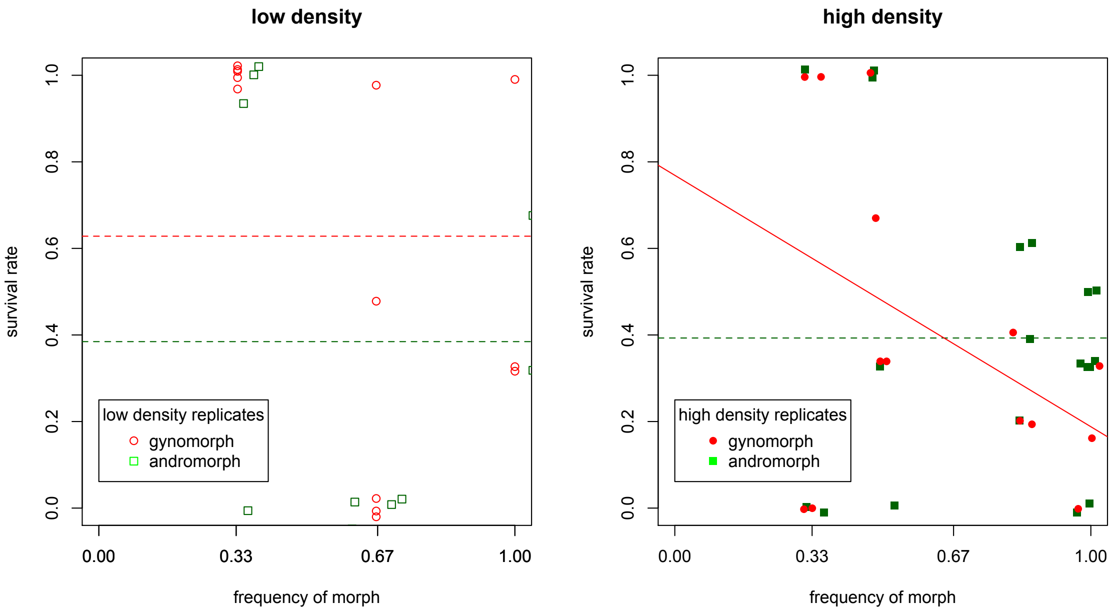

Figure 5.4 Survival of *Ischnura ramburii* andromorphs and gynomorphs at high and low densities. Gynomorph fitness was frequency-dependent in high-density conditions.

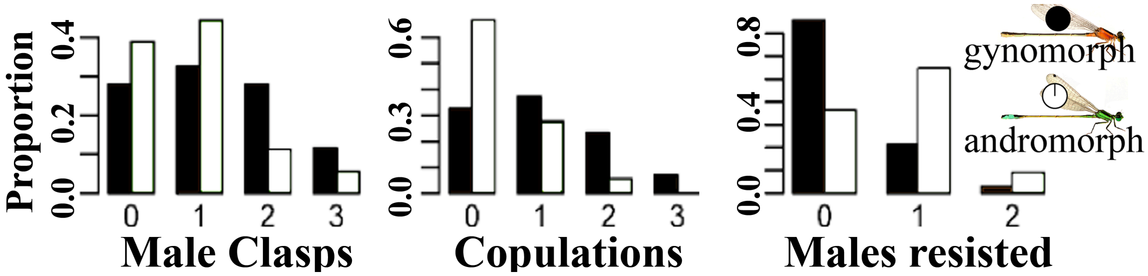

Figure 5.5 Results of dust-recapture experiments of male-female interaction in *I. ramburii*. Results depicted are pooled from high and low-density treatmentst; see Results section for details and analyses.

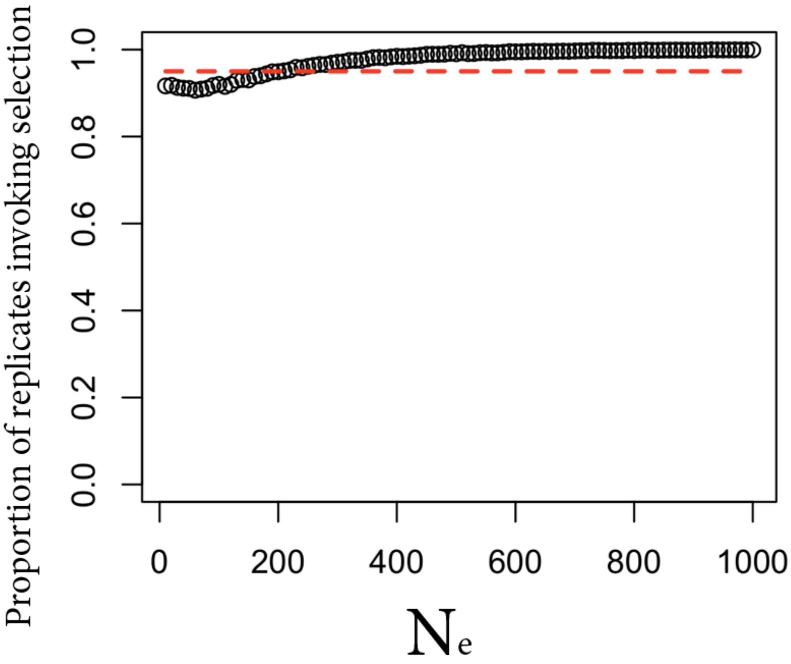

Figure 5.S1. Outcomes of forward simulations of genetic drift using Kimura's diffusion model, starting from historic morph frequencies and using methods described in the text (N=100,000 replicates per effective populations size, Ne. Dashed line=0.95). Replicates invoking selection were those in which drift alone did not produce morph frequency changes $\geq$ those observed within Hawaii populations of *Ischnura ramburii*. Results are depicted for the single locality that contained andromorphs in historic surveys. Similar results were obtained from historically monomorphic populations under the conservative assumption that historic andromorph frequencies = 1/ (1+ # gynomorphs reported).

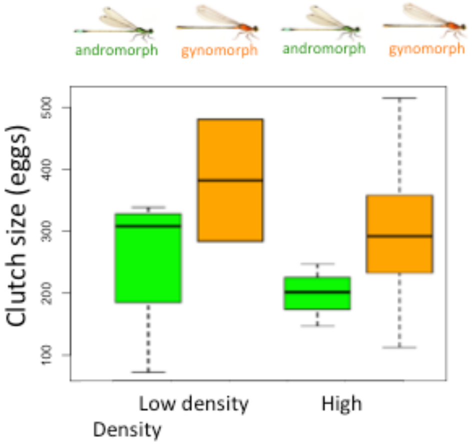

Figure 5.S2. Fecundities of *Ischnura ramburii* andromorphs and gynomorphs from high vs. low-density mesocosm experiments; illustrations from Abbott 2012.

Table 5.S1. Museum data used to estimate morph frequencies to augment published data and field data

species	date collected	country	locality	collected by	morph	collection
I. ramburii	10/24/16	Alabama	Mobile		male	American Museum of Natural History
I. ramburii	6/9/94	Arizona	Agua Caliente Park, E edge Tucson	Dunkle	orange female	Breckenridge Field Lab
I. ramburii	6/9/94	Arizona	Agua Caliente Park, E edge Tucson	Dunkle	orange female	Breckenridge Field Lab
I. ramburii	6/9/94	Arizona	Agua Caliente Park, E edge Tucson	Dunkle	olive female	Breckenridge Field Lab
I. ramburii	6/9/94	Arizona	Agua Caliente Park, E edge Tucson	Dunkle	male	Breckenridge Field Lab
I. ramburii	6/9/94	Arizona	Agua Caliente Park, E edge Tucson	Dunkle	orange female	Breckenridge Field Lab
I. ramburii	6/9/94	Arizona	Agua Caliente Park, E edge Tucson	Dunkle	male	Breckenridge Field Lab
I. ramburii	6/9/94	Arizona	Agua Caliente Park, E edge Tucson	Dunkle	orange female	Breckenridge Field Lab
I. ramburii	5/26/04	Arkansas	Stone County; pond 3 miles east of Optimus off hwy 5; Ozark national forest	Abbott	mimetic female	Breckenridge Field Lab
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	mimetic female	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	mimetic female	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	mimetic female	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville

Table 5.S1, cont.

I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	mimetic female	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	mimetic female	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	mimetic female	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	male	Gainesville
I. ramburii	8/1/88	Bermuda	Warwick Pond	Dunkle	mimetic female	Gainesville
I. ramburii	6/10/09	Caymans	West Indes, Cayman Islands, Georgetown	Gilleut	male	Gainesville
I. ramburii	2/1/65	Chile	Antofagasta Province, Quillaga	Cook	male	Cook's private collection
I. ramburii	5/23/09	Colombia	San Andres Island	Rentz	male	California Academy of Sciences*
I. ramburii	8/1/99	Connecticut	Fairfield County, Stratford, Lordship Marsh.	sibley	male	Gainesville
I. ramburii	7/20/89	Costa Rica	San Jose Province, Pond in Hacienda Lindora, 4km sw of Santa Ana	C. Esquivel	male	Gainesville
I. ramburii	7/20/89	Costa Rica	San Jose Province, Pond in Hacienda Lindora, 4km sw of Santa Ana	C. Esquivel	male	Gainesville
I. ramburii	7/20/89	Costa Rica	San Jose Province, Pond in Hacienda Lindora, 4km sw of Santa Ana	C. Esquivel	male	Gainesville
I. ramburii	10/1/02	Costa Rica	puentarenas province, coto brus canton, san joaquin wetlands, 1.5miles east of San Vito	sibley	olive female	Gainesville
I. ramburii	3/21/65	Costa Rica	Provencia de cartago, Turrialba	Janzen	male	California Academy of Sciences*
I. ramburii	3/23/65	Costa Rica	Provencia de Puntarenas: Hacienda Tobago 15km SE Canas	Janzen	mimetic female	California Academy of Sciences*
I. ramburii	3/21/65	Costa Rica	Provencia de cartago, Turrialba	Janzen	orange female	California Academy of Sciences*
I. ramburii	3/21/65	Costa Rica	Provencia de cartago, Turrialba	Janzen	orange female	California Academy of Sciences*

Table 5.S1, cont.

ramburii	3/21/65	Costa Rica	Provencia de cartago, Turrialba	Janzen	mimetic female	California Academy of Sciences*
I. ramburii	3/21/65	Costa Rica	Provencia de cartago, Turrialba	Janzen	mimetic female	California Academy of Sciences*
I. ramburii	3/21/65	Costa Rica	Provencia de cartago, Turrialba	Janzen	male	California Academy of Sciences*
I. ramburii	3/21/65	Costa Rica	Provencia de cartago, Turrialba	Janzen	orange female	California Academy of Sciences*
I. ramburii	6/1/60	Cuba	Las Villas Province; Trinidata Mts. near Salto del Hanabanilla	westfall	not recorded	Gainesville
I. ramburii	6/1/60	Cuba	Las Villas Province; Trinidata Mts. near Salto del Hanabanilla	westfall	not recorded	Gainesville
I. ramburii	6/1/60	Cuba	Las Villas Province; Trinidata Mts. near Salto del Hanabanilla	westfall	not recorded	Gainesville
I. ramburii	6/1/59	Cuba	Cuba, Las Villas Province, Trinidad mountains near salto del hanabanilla	Westfall	mimetic female	Gainesville
I. ramburii	6/1/59	Cuba	Cuba, Las Villas Province, Trinidad mountains near salto del hanabanilla	Westfall	mimetic female	Gainesville
I. ramburii	6/1/59	Cuba	Cuba, Las Villas Province, Trinidad mountains near salto del hanabanilla	Westfall	mimetic female	Gainesville
I. ramburii	5/1/59	Cuba	Cuba, Las Villas Province, Atkins Botanical Garden at Soledad	westfall	orange female	Gainesville
I. ramburii	5/1/59	Cuba	Cuba, Las Villas Province, Atkins Botanical Garden at Soledad	westfall	mimetic female	Gainesville
I. ramburii	5/1/59	Cuba	Cuba, Las Villas Province, Atkins Botanical Garden at Soledad	westfall	male	Gainesville
I. ramburii	5/1/59	Cuba	Cuba, Las Villas Province, Atkins Botanical Garden at Soledad	westfall	male	Gainesville
I. ramburii	5/1/59	Cuba	Cuba, Las Villas Province, Atkins Botanical Garden at Soledad	westfall	mimetic female	Gainesville
I. ramburii		Cuba	Havana		orange female	American Museum of Natural History

Table 5.S1, cont.

I. ramburii		Cuba	Havana		male	American Museum of Natural History
I. ramburii		Cuba	Havana		male	American Museum of Natural History
I. ramburii		Cuba	Havana		male	American Museum of Natural History
I. ramburii		Cuba	Havana		male	American Museum of Natural History
I. ramburii		Cuba	Havana		orange female	American Museum of Natural History
I. ramburii		Cuba	Havana		orange female	American Museum of Natural History
I. ramburii	9/6/13	Cuba	cabanas		orange female	American Museum of Natural History
I. ramburii		Cuba	Havana		orange female	American Museum of Natural History
I. ramburii		Cuba	Havana		mimetic female	American Museum of Natural History
I. ramburii	4/14/09	Cuba	Havana		orange female	American Museum of Natural History
I. ramburii	5/25/15	Dominican Republic			olive female	American Museum of Natural History
I. ramburii	2/1/97	Ecuado	Imabura prov. Lita; 46km east of Lita, rocky stream	Mauffray	male	Gainesville
I. ramburii	2/1/97	Ecuador	Imabura prov. pool 1.5km S of Salinas	Tennessen	mimetic female	Gainesville
I. ramburii	2/1/97	Ecuador	Imabura prov. pool 1.5km S of Salinas	Tapia	male	Gainesville
I. ramburii	3/31/53	Eleuthera Island			male	American Museum of Natural History
I. ramburii	4/1/46	Florida	Lower Mantecumbe Key	Cook	male	Cook's private collection
I. ramburii	10/1/73	Florida	Fort Walton, Okaloosa county	Cook	male	Cook's private collection
I. ramburii	10/1/73	Florida	Fort Walton, Okaloosa county	Cook	orange female	Cook's private collection
I. ramburii	10/1/73	Florida	Okaloosa County, Fort Walton	Cook	male	Cook's private collection
I. ramburii	10/1/73	Florida	Okaloosa County, Fort Walton	Cook	orange female	Cook's private collection
I. ramburii	9/20/80	Florida	Alachua County; pond in NE Gainesville	Dunkle	olive female	Breckenridge Field Lab
I. ramburii	9/20/80	Florida	Alachua County; pond in NE Gainesville	Dunkle	olive female	Breckenridge Field Lab
I. ramburii	9/20/80	Florida	Alachua County; pond in NE Gainesville	Dunkle	olive female	Breckenridge Field Lab
I. ramburii	3/23/88	Florida	Sarasota County; Mote Marine lab @ Sarasota, Florida	Moulton	male	Breckenridge Field Lab

Table 5.S1, cont.

I. ramburii	3/23/88	Florida	Sarasota County; Mote Marine lab @ Sarasota, Florida	Moulton	male	Breckenridge Field Lab
I. ramburii	3/21/88	Florida	Sarasota County; Mote Marine lab @ Sarasota, Florida	Moulton	female	Breckenridge Field Lab
I. ramburii	3/8/88	Florida	Alachua County; SW Gainesville	Dunkle	orange female	Breckenridge Field Lab
I. ramburii	3/8/88	Florida	Alachua County; SW Gainesville	Dunkle	orange female	Breckenridge Field Lab
I. ramburii		Florida			male	American Museum of Natural History
I. ramburii	3/21/21	Florida			olive female	American Museum of Natural History
I. ramburii	3/21/21	Florida			olive-orange female	American Museum of Natural History
I. ramburii	3/29/21	Florida	moore haven	H. Williamson	male	American Museum of Natural History
I. ramburii	3/29/21	Florida	moore haven	H. Williamson	male	American Museum of Natural History
I. ramburii	3/29/21	Florida	moore haven	H. Williamson	male	American Museum of Natural History
I. ramburii	3/30/21	Florida	moore haven	H. Williamson	male	American Museum of Natural History
I. ramburii	3/29/21	Florida	moore haven	H. Williamson	male	American Museum of Natural History
I. ramburii	3/29/21	Florida	moore haven	H. Williamson	male	American Museum of Natural History
I. ramburii		Florida	orlando		olive-orange female	American Museum of Natural History
I. ramburii		Florida	orlando		male	American Museum of Natural History
I. ramburii		Florida	Levy Co.		male	American Museum of Natural History
I. ramburii		Florida			olive-orange female	American Museum of Natural History
I. ramburii	7/12/32	Florida	Miami		male	American Museum of Natural History
I. ramburii	7/12/32	Florida	Miami		male	American Museum of Natural History
I. ramburii	9/6/69	Georgia	Harris County, Hamilton Franklin D. Roosevelt State Park	Bartholemew	mimetic female	California Academy of Sciences*
I. ramburii	7/1/98	guadeloupe	Basse-Terre, 2.5 km N of Deshaies at La Grande Anse. brackish lake	sibley	orange female	Gainesville
I. ramburii	7/1/98	guadeloupe	Basse-Terre, 2.5 km N of Deshaies at La Grande Anse. brackish lake	sibley	orange female	Gainesville

Table 5.S1, cont.

I. ramburii	7/1/98	guadeloupe	Basse-Terre, 4km nw of Saint-Sauveur at Grant Etang. large mountain freshwater lake	sibley	mimetic female	Gainesville
I. ramburii	4/1/01	Guadeloupe	Basse-Terre, Cascade aux Exrevisses	stange	male	Gainesville
I. ramburii	4/1/01	Guadeloupe	Basse-Terre, Cascade aux Exrevisses	stange	mimetic female	Gainesville
I. ramburii	7/1/98	Guadeloupe	Basse-Terre, 4km nw of Saint-Sauveur at Grant Etang. large mountain freshwater lake	sibley	male	Gainesville
I. ramburii	7/1/98	Guadeloupe	Basse-Terre, 5km nne of deshaies at fort royal.	sibley	male	Gainesville
I. ramburii	7/1/98	Guadeloupe	Basse-Terre, 4km nw of Saint-Sauveur at Grant Etang. large mountain freshwater lake	sibley	mimetic female	Gainesville
I. ramburii	7/27/98	Guadeloupe	Basse-Terre, 4km nw of Saint-Sauveur at Grant Etang. large mountain freshwater lake	sibley	orange female	Gainesville
I. ramburii	7/1/98	Guadeloupe	Basse-Terre, 2.5 km N of Deshaies at La Grande Anse. brackish lake	sibley	olive female	Cook's private collection
I. ramburii	6/1/64	Guatemala	puerto Barrios	thompson	mimetic female	Gainesville
I. ramburii	7/1/64	Guatemala	puerto Barrios	thompson	male	Gainesville
I. ramburii	8/1/64	Guatemala	puerto Barrios	thompson	male	Gainesville
I. ramburii	9/1/64	Guatemala	puerto Barrios	thompson	olive female	Gainesville
I. ramburii	8/1/67	Guatemala	lago de atitlan at panajachel 5100'	paulson	male	Gainesville
I. ramburii	9/1/67	Guatemala	lago de atitlan at panajachel 5100'	paulson	mimetic female	Gainesville
I. ramburii	7/1/86	Guatemala	finca la herradura (Escuintla dept.)	dix	male	Gainesville
I. ramburii	8/1/84	Guatemala	finca la herradura (Escuintla dept.)	montalbau	male	Gainesville
I. ramburii	3/1/80	Guatemala	laguna de calderas	donnelly	orange female	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville

Table 5.S1, cont.

I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	mimetic female	Gainesville
I. ramburii	7/1/83	Guatemala	flores (el peten)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	mimetic female	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	orange female	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	mimetic female	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	mimetic female	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	olive female	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	mimetic female	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	mimetic female	Gainesville
I. ramburii	7/1/62	Guatemala	lake atitlan (1560m)	donnelly	mimetic female	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	olive female	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	olive female	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	7/1/62	Guatemala	lk. nr san francisco de sales nr volcan (1800m) pacaya	donnelly	male	Gainesville
I. ramburii	8/1/62	Guatemala	lk. nr san francisco de sales nr volcan (1800m) pacaya	donnelly	mimetic female	Gainesville
I. ramburii	8/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville

Table 5.S1, cont.

I. ramburii	8/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	8/1/62	Guatemala	lake atitlan (1560m)	donnelly	male	Gainesville
I. ramburii	9/1/64	Guatemala	los aposentos (1700m)	donnelly	male	Gainesville
I. ramburii	9/1/64	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	9/1/64	Guatemala	tikal, aguada@camp	donnelly	male	Gainesville
I. ramburii	8/1/64	Guatemala	lk. nr san francisco de sales nr volcan (1800m) pacaya	donnelly	male	Gainesville
I. ramburii	8/1/64	Guatemala	tikal, main aguada	dix	mimetic female	Gainesville
I. ramburii	8/1/64	Guatemala	izabal 5dm s. san felipe	donnelly	male	Gainesville
I. ramburii	3/2/22	Haiti			male	American Museum of Natural History
I. ramburii	4/1/78	Hawaii	Oahu, Pearl City	Ginoza	male	UH manoa collection (Rubinoff)
I. ramburii	4/1/78	Hawaii	Oahu, Pearl City	Ginoza	orange female	UH manoa collection (Rubinoff)
I. ramburii	10/24/76	Hawaii	Oahu, Honolulu Zoo	Arakaki	male	UH manoa collection (Rubinoff)
I. ramburii	10/12/78	Hawaii	Oahu, Pearl City Sweet potatoe	Chun	male	UH manoa collection (Rubinoff)
I. ramburii	10/13/77	Hawaii	Oahu, UH Manoa	Osaki	male	UH manoa collection (Rubinoff)
I. ramburii	5/7/73	Hawaii	Oahu, Pearl City	Beer	male	UH manoa collection (Rubinoff)
I. ramburii	5/7/73	Hawaii	Oahu, Pearl City	Beer	male	UH manoa collection (Rubinoff)
I. ramburii	2/16/80	Hawaii	Oahu, Manoa	Robin	male	UH manoa collection (Rubinoff)
I. ramburii	9/19/78	Hawaii	Oahu, Pearl City near pond	Suyeoka	male	UH manoa collection (Rubinoff)
I. ramburii	1/27/80	Hawaii	Oahu, Manoa	Yamamoto	male	UH manoa collection (Rubinoff)
I. ramburii	11/21/77	Hawaii	Oahu? "P.C.I.F." (Pearl City?)	Kobashigawa	male	UH manoa collection (Rubinoff)
I. ramburii	11/21/77	Hawaii	Oahu, Pearl City	Kobashigawa	male	UH manoa collection (Rubinoff)
I. ramburii	11/21/77	Hawaii	Oahu, Pearl City	Kobashigawa	male	UH manoa collection (Rubinoff)
I. ramburii	11/21/77	Hawaii	Oahu, Pearl City	Kobashigawa	male	UH manoa collection (Rubinoff)
I. ramburii	9/5/77	Hawaii	Oahu, Kailua "Bidens pilosa (misidentified?)	Dunlap	male	UH manoa collection (Rubinoff)
I. ramburii	11/18/80	Hawaii	Oahu, Pearl City	Pratt	male	UH manoa collection (Rubinoff)
I. ramburii	12/4/77	Hawaii	Oahu, Pearl City	Tanou	male	UH manoa collection (Rubinoff)

Table 5.S1, cont.

I. ramburii	10/25/75	Hawaii	Oahu, UH quarry	Chin	male	UH manoa collection (Rubinoff)
I. ramburii	10/18/76	Hawaii	Oahu, Honolulu Zoo	McKeown	male	UH manoa collection (Rubinoff)
I. ramburii	2/24/78	Hawaii	Oahu, Waiau Swamp	Watanabe	male	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	orange female	UH manoa collection (Rubinoff)
I. ramburii	1/28/74	Hawaii	Oahu, UH Quarry	Baker	orange female	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	orange female	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Pahoa	Santo	orange female	UH manoa collection (Rubinoff)
I. ramburii	9/26/77	Hawaii	Oahu, Pearl City "Colocasia asculenta" (misidentified)	Dunlap	orange female	UH manoa collection (Rubinoff)
I. ramburii	9/26/77	Hawaii	Oahu, Pearl City, Saran House	Scharf	olivish-orange female	UH manoa collection (Rubinoff)
I. ramburii	9/26/77	Hawaii	Oahu, Pearl City, grass	Nakamura	orange female	UH manoa collection (Rubinoff)
I. ramburii	11/21/77	Hawaii	Oahu, Pearl City	Kobashigawa	orange female	UH manoa collection (Rubinoff)
I. ramburii	11/28/74	Hawaii	Oahu, Moanalua	Teruya	orange female	UH manoa collection (Rubinoff)
I. ramburii	9/9/75	Hawaii	Oahu, Kaneohe	Sakamoto	orange female	UH manoa collection (Rubinoff)
I. ramburii	11/12/78	Hawaii	Oahu, Pearl City, tall grass	Chun	orange female	UH manoa collection (Rubinoff)
I. ramburii	12/1/74	Hawaii	Oahu, Pearl City	Nakatani	orange female	UH manoa collection (Rubinoff)
I. ramburii	8/9/75	Hawaii	Oahu, Kaneohe	Sakamoto	orange female	UH manoa collection (Rubinoff)
I. ramburii	2/6/78	Hawaii	Oahu, Pearl City Instr Fac. "Ipomea Aquatica"	Watanabe	orange female	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	orange female	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Oahu, Pearl City	Santo	orange female	UH manoa collection (Rubinoff)
I. ramburii	3/1/74	Hawaii	Oahu	Beardsly	orange female	UH manoa collection (Rubinoff)
I. ramburii	3/8/74	Hawaii	Oahu, UH campus	Uehara	olive female	UH manoa collection (Rubinoff)
I. ramburii	9/17/73	Hawaii	Oahu, Manoa	Hanada	olive female	UH manoa collection (Rubinoff)
I. ramburii	6/8/86	Hawaii	Oahu, Manoa Hawaii Taro Farm	Ashek	orange female	UH manoa collection (Rubinoff)
I. ramburii	6/8/86	Hawaii	Oahu, Manoa Hawaii Taro Farm	Ashek	olive female	UH manoa collection (Rubinoff)
I. ramburii	3/1/86	Hawaii	Oahu, Manoa Valley	Hiranaka	orange female	UH manoa collection (Rubinoff)
I. ramburii	4/20/86	Hawaii	Oahu, Nuuanu? Pali Stream	Iwamoto	olive female	UH manoa collection (Rubinoff)

Table 5.S1, cont.

I. ramburii	9/17/73	Hawaii	Oahu, Manoa	Hanada	orange female	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	male	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Pahoa	Santo	male	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	male	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	male	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	male	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Pahoa	Santo	male	UH manoa collection (Rubinoff)
I. ramburii	11/26/72	Hawaii	Big Island, UH Quarry	Dennis	male	UH manoa collection (Rubinoff)
I. ramburii	5/1/71	Hawaii	Oahu, Pearl City	Nichols	male	UH manoa collection (Rubinoff)
I. ramburii	4/11/71	Hawaii	Big Island, Kapoho	Santo	male	UH manoa collection (Rubinoff)
I. ramburii	5/2/86	Hawaii	Oahu, UH Prawn Farm	Iwamoto	male	UH manoa collection (Rubinoff)
I. posita	1/22/48	Hawaii	Molokai, Hawaii - Keawanui	Krauss	male	Bishop museum collection
I. posita	1/23/48	Hawaii	Molokai, Hawaii - Keawanui	Krauss	female	Bishop museum collection
I. posita	8/13/58	Hawaii	Oahu, Helemano	Quate	female	Bishop museum collection
I. posita	5/1/56	Hawaii	Maui, Palekea	Grassitt	male	Bishop museum collection
I. posita	8/13/58	Hawaii	Oahu, Helemano	Quate	female	Bishop museum collection
I. posita	10/30/92	Hawaii	Oahu	Smith	female	Bishop museum collection
I. posita	9/4/47	Hawaii	Molokai, Halawa valley	Krauss	male	Bishop museum collection
I. posita	9/4/47	Hawaii	Molokai, Halawa valley	Krauss	male	Bishop museum collection
I. posita	9/4/47	Hawaii	Molokai, Halawa valley	Krauss	male	Bishop museum collection
I. posita	9/4/47	Hawaii	Molokai, Halawa valley	Krauss	male	Bishop museum collection
I. posita	9/4/47	Hawaii	Molokai, Halawa valley	Krauss	female	Bishop museum collection
I. ramburii	9/8/88	Hawaii	Kauai, Nat. Trop. Bot. Garden	Miller	male	Bishop museum collection
I. posita	3/18/84	Hawaii	Oahu, Makiki stream 320ft elevation	Perreira	female	Bishop museum collection
I. posita	2/1/37	Hawaii	Oahu, Manoa	FXW	male	Bishop museum collection
I. ramburii	2/15/82	Hawaii	Oahu, Kauaina Marsh	Ching	orange female	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, sea level	Gagne?	orange female	Bishop museum collection

Table 5.S1, cont.

I. ramburii	6/9/95	Hawaii	Big Island, Kaphoh Crater Green Lake, Puna	Polhemus	male	Bishop museum collection
I. ramburii	8/31/95	Hawaii	Molokai, mouth of pelekunu stream	Polhemus	orange female	Bishop museum collection
I. ramburii	8/30/95	Hawaii	Molokai, Palaau wetland 3 mi west of Kaunakakai, sea level mixohaline pond at molokai sea farms aquaculture project	Polhemus	olive-orange female	Bishop museum collection
I. ramburii	8/30/95	Hawaii	Molokai, Kahakaia pond national wildlife refuge e of Kaunakakai sea level	Polhemus	olive-orange female	Bishop museum collection
I. ramburii	4/27/95	Hawaii	Lanai, ponds at Lanai city wastewater treatment plant near lanai city, 1800ft	Polhemus	male	Bishop museum collection
I. ramburii	8/17/95	Hawaii	Oahu, Kawainui Marsh, Kilua	Preston	male	Bishop museum collection
I. ramburii	4/27/95	Hawaii	Lanai, ponds at Lanai city wastewater treatment plant near lanai city, 1800ft	Polhemus	orange female	Bishop museum collection
I. ramburii	8/17/95	Hawaii	Oahu, ponds at BWS water facility upper nuuanu valley 800ft	Preston	olive-orange female	Bishop museum collection
I. ramburii	8/17/95	Hawaii	Oahu, Kawainui Marsh, Kailua	Polhemus	orange female	Bishop museum collection
I. ramburii	6/7/73	Hawaii	Oahu, salt lake (aliapakai) 50ft - specimen in fragments	Gagne	male	Bishop museum collection
I. ramburii	6/2/74	Hawaii	Oahu, sea level, w loc pearl Harbor	Gagne	male	Bishop museum collection
I. ramburii	3/3/79	Hawaii	Oahu, Pearl city - specimen broken	Uchida	m	Bishop museum collection
I. ramburii	3/3/79	Hawaii	Oahu, Pearl City	Uchida	m	Bishop museum collection
I. ramburii	9/26/80	Hawaii	Oahu, Koolau mts	Howarth	olive-orange female	Bishop museum collection
I. ramburii	8/7/82	Hawaii	Big Island, Waipio Valley, 0-50m	Howarth	olive female	Bishop museum collection
I. ramburii	8/8/82	Hawaii	Big Island, Waipio Valley, 0-50m	Howarth	orange female	Bishop museum collection
I. ramburii	8/9/82	Hawaii	Big Island, Waipio Valley, 0-50m	Howarth	male	Bishop museum collection
I. ramburii	8/10/82	Hawaii	Big Island, Waipio Valley, 0-50m	Howarth	male	Bishop museum collection
I. ramburii	3/7/73	Hawaii	Big Island, Hilo, Wailoa SP	Howarth	male	Bishop museum collection

Table 5.S1, cont.

I. ramburii	2/2/74	Hawaii	Oahu, Pearl City	Howarth	olive-orange female	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, Pearl City	Howarth	male	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	male	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	female	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	male	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	male	Bishop museum collection
I. ramburii	3/7/73	Hawaii	Big Island, Hilo, Wailoa State park	Harwood	male	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	male	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	female	Bishop museum collection
I. ramburii	9/15/87	Hawaii	Oahu, Moanalua Gardens	Howarth	male	Bishop museum collection
I. ramburii	9/15/87	Hawaii	Oahu, Moanalua Gardens	Howarth	female	Bishop museum collection
I. ramburii	9/15/87	Hawaii	Oahu, Moanalua Gardens	Howarth	male	Bishop museum collection
I. ramburii	9/15/87	Hawaii	Oahu, Moanalua Gardens	Howarth	female	Bishop museum collection
I. ramburii	9/15/87	Hawaii	Oahu, Moanalua Gardens	Howarth	orange female	Bishop museum collection
I. ramburii	11/1/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	male	Bishop museum collection
I. ramburii	11/2/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	male	Bishop museum collection
I. ramburii	11/3/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	male	Bishop museum collection
I. ramburii	11/4/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	male	Bishop museum collection
I. ramburii	11/5/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	olive-orange female	Bishop museum collection
I. ramburii	11/6/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	orange female	Bishop museum collection
I. ramburii	11/7/80	Hawaii	Oahu, Barber's point, flying nr limestone quarry seeps	Gagne	orange female	Bishop museum collection

Table 5.S1, cont.

I. ramburii	11/8/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	orange female	Bishop museum collection
I. ramburii	9/1/80	Hawaii	Oahu, Barber's point, flying near seepages in limestone quarry	Gagne	orange female	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	orange female	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	orange female	Bishop museum collection
I. ramburii	3/3/79	Hawaii	Oahu, Pearl City	Uchida	orange female	Bishop museum collection
I. ramburii	3/3/79	Hawaii	Oahu, Pearl City	Uchida	orange female	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	orange female	Bishop museum collection
I. ramburii	2/2/74	Hawaii	Oahu, W. loch, Pearl Harbor	Howarth	orange female	Bishop museum collection
I. posita	6/16/62	Hawaii	Molokai, Halawa valley - broken	Yano	indeterminable	Bishop museum collection
I. posita	6/16/62	Hawaii	Molokai, Halawa valley - broken	Yano	indeterminable	Bishop museum collection
I. posita	8/30/95	Hawaii	Molokai, Palaau wetland 3 mi west of Kaunakakai, sea level mixohaline pond at molokai sea farms aquaculture project	Polhemus	female	Bishop museum collection
I. posita	1/2/76	Hawaii	Oahu, Nuuanu valley	Howarth	male	Bishop museum collection
I. posita	7/1/77	Hawaii	Oahu, Koolau mts waikane hakipuu	Thomas	male	Bishop museum collection
I. posita	12/2/56	Hawaii	Oahu, Kailua -specimen in pieces	Gressitt	indeterminable	Bishop museum collection
I. posita	3/16/41	Hawaii	Oahu, honolulu	Kondo	female	Bishop museum collection
I. posita	3/16/41	Hawaii	Oahu, honolulu	Kondo	female	Bishop museum collection
I. ramburii		Hawaii?	Oahu, Laie	Sekimura	olive female	UH manoa collection (Rubinoff)
I. ramburii	8/1/92	Honduras	El Zamorano	stange	male	Gainesville
I. ramburii	8/1/92	Honduras	El Zamorano	stange	olive female	Gainesville
I. ramburii	1/1/20	Jamaica	Montego Bay, St. Jathee		male	American Museum of Natural History
I. posita	10/1/03	Kentucky	Edmonson county, mcnp	Cook	male	Cook's private collection
I. ramburii	5/20/92	Louisianna	Allen Parish; Calcasieu River @ LA 26 Bridge	Abbott	olive female	Breckenridge Field Lab

Table 5.S1, cont.

I. ramburii	5/20/92	Louisianna	Allen Parish; Calcasieu River @ LA 26 Bridge	Abbott	mimetic female	Breckenridge Field Lab
I. ramburii	5/20/92	Louisianna	Allen Parish; Calcasieu River @ LA 26 Bridge	Abbott	male	Breckenridge Field Lab
I. ramburii	7/1/92	Mexico	Veracruz, pond near Rio la Palma, 8km N of Sontecomapan	Tennessen	male	Gainesville
I. ramburii	7/1/92	Mexico	Veracruz, sontecomapan; N on road to Los Tuxlas Rio La Palma/ond and roadside spring pool	Mauffray	male	Gainesville
I. ramburii	7/1/92	Mexico	veracruz, pond near rio la palma, 8km N of Sontecomapan	Tennessen	male	Gainesville
I. ramburii	7/1/92	Mexico	veracruz, pond near rio la palma, 8km N of Sontecomapan	Tennessen	male	Gainesville
I. ramburii	7/1/92	Mexico	veracruz, pond near rio la palma, 8km N of Sontecomapan	Tennessen	orange female	Gainesville
I. ramburii	9/1/43	Mexico	San Jose del Cabo	G. Eisen	orange female	California Academy of Sciences*
I. ramburii	9/1/43	Mexico	San Jose del Cabo	G. Eisen	orange female	California Academy of Sciences*
I. ramburii	9/1/43	Mexico	San Jose del Cabo	G. Eisen	olive female	California Academy of Sciences*
I. ramburii	9/26/48	Mexico	15 miles N Pena, Nayarit	E.S. Ross	male	California Academy of Sciences*
I. ramburii	9/26/48	Mexico	15 miles N Pena, Nayarit	E.S. Ross	male	California Academy of Sciences*
I. ramburii		Mexico	Sinaloa, Mazatlan	Noonan	male	California Academy of Sciences*
I. ramburii		Mexico	Sinaloa, Mazatlan	Noonan	male	California Academy of Sciences*
I. ramburii		Mexico	Mazatlan		orange female	American Museum of Natural History
I. ramburii	6/17/05	Mississippi	carroll county@county road 77 crossing, ca 6 miles ENE of North Carrollton	Krotzer	male	Gainesville
I. ramburii	3/30/06	Mississippi	clairborne county. borrow ponds adjacent to mississippi river, off main grand gulf road, ca 1.5 miles ssw of grand gulf	Krotzer	male	Gainesville

Table 5.S1, cont.

I. ramburii	5/5/08	Mississippi	Oxbow Lake, by Levee road, 3.5 mile sw glen allan	Thomas	male	Gainesville
I. ramburii	7/1/06	Mississippi	Lamar County, inflow to little black creek water park, courtney road crossing, ca 4miles e of baxterville	Krotzer	male	Gainesville
I. ramburii	6/5/05	Mississippi	Leflore county, catfish ponds just E of County road 552, ca 4miles s of morgan city	Krotzer	mimetic female	Gainesville
I. ramburii	6/5/05	Mississippi	Leflore county, catfish ponds just E of County road 552, ca 4miles s of morgan city	Krotzer	male	Gainesville
I. ramburii	7/1/06	Mississippi	Perry county, lake perry at lake perry road, desoto National forest 3 miles sse of beaumont	Krotzer	male	Gainesville
I. ramburii	5/5/05	Mississippi	Yazoo county off US hwy49W, ca .5miles W of Yazoo City, east end of whites lane	Daigle	male	Gainesville
I. ramburii	5/5/05	Mississippi	Washington County, cox ponds, yazoo nwr, cox road, ca 2miles NE or Erwin	Krotzer	olive female	Gainesville
I. ramburii	5/5/05	Mississippi	Washington County, cox ponds, yazoo nwr, cox road, ca 2miles NE or Erwin	Krotzer	olive female	Gainesville
I. ramburii	7/5/05	Mississippi	Tate County, Hickahala Creek at Arkabutla Road, ca 2 miles WSW of Coldwater	Krotzer	male	Gainesville
I. ramburii	6/1/05	Mississippi	Tallahatchie NWR, just S of MS hwy8, ca. 2.5 miles ene of philipp	Krotzer	male	Gainesville
I. ramburii	5/1/05	Mississippi	Big Sunflower county, big sunflower river at ms hwy 442 ca 2 miles wsw of doddsville	Krotzer	female	Gainesville
I. ramburii	9/1/45	New Jersey	Davis Lake, Cape May	Beatty	male	Cook's private collection
I. ramburii	9/1/45	New Jersey	Davis Lake, Cape May	Beatty	male	Cook's private collection
I. ramburii	6/16/17	New Jersey	Lakehurst		male	American Museum of Natural History
I. ramburii	9/23/16	New Jersey	Lakehurst		male	American Museum of Natural History

Table 5.S1, cont.

I. ramburii	9/16/16	New Jersey	Lakehurst		male	American Museum of Natural History
I. ramburii	8/1/95	New Jersey, Cape May Co.	Dennis Township Restoration site#2	Kauffman	orange female	Gainesville
I. ramburii	8/1/95	New Jersey, Cape May Co.	Dennis Township Restoration site#2	Kauffman	male	Gainesville
I. posita	6/1/46	New Jersey, Cape May Co.		G.H. Beatty	male	Cook's private collection
I. posita	6/1/46	New Jersey, Cape May Co.		G.H. Beatty	female	Cook's private collection
I. ramburii	7/1/51	New York	Suffolk Co. Westhampton, Long Island	Latham	male	Gainesville
I. ramburii	7/1/51	New York	Suffolk Co. Westhampton, Long Island	Latham	olive female	Gainesville
I. ramburii	7/1/51	New York	Suffolk Co. Westhampton, Long Island	Latham	olive female	Gainesville
I. ramburii	7/1/51	New York	Suffolk Co. Westhampton, Long Island	Latham	olive female	Gainesville
I. ramburii	7/1/97	New York	Suffolk Co. Westhampton, Long Island	Latham	male	Gainesville
I. ramburii	5/3/03	North Carolina	route 17 bypass, hertford	cuyler	orange female	Gainesville
I. ramburii	11/1/94	North Carolina	Ditch, rt. 264, rose bay	Cuyler	mimetic female	Gainesville
I. ramburii	8/1/94	North Carolina	High Rock Lake, rt. 47, 1.7 miles of Jct. Rt. 8 (sic)	Cuyler	male	Gainesville
I. ramburii	8/1/94	North Carolina	High Rock Lake, rt. 47, 1.7 miles of Jct. Rt. 8 (sic)	cuyler	mimetic female	Gainesville
I. ramburii	5/1/93	North Carolina	Stanly county North Bank, Rocky River 1 mile south, Norwood	Cuyler	olive female	Gainesville
I. ramburii	9/1/01	North Carolina	ICW access area, rte. 210	Cuyler	male	Cook's private collection
I. ramburii	8/20/98	Oahu	Honolulu, Moanalua Gardens Picnic area, moanalua stream	Mauffray	male	Gainesville
I. ramburii	7/21/63	Oklahoma	Pittsburg County; McAlister, 1 mile East	Bick	male	Breckenridge Field Lab
I. ramburii	11/1/55	Puerto Rico	Laguna Cartegena	Cross	olive female	Gainesville
I. ramburii	11/1/55	Puerto Rico	Laguna Cartegena	Cross	male	Gainesville
I. ramburii	8/1/63	Puerto Rico	Mayaguez, Agric. Exper. Station	donnelly	male	Gainesville
I. ramburii	8/1/63	Puerto Rico	Mayaguez, Agric. Exper. Station	donnelly	mimetic female	Gainesville

Table 5.S1, cont.

I. ramburii	11/1/78	Puerto Rico	Ponce	Macheli	orange female	Cook's private collection
I. ramburii	11/10/25	Puerto Rico	aibonito		male	American Museum of Natural History
I. ramburii	3/4/14	Puerto Rico	Arecibo		male	American Museum of Natural History
I. ramburii	11/14/25	Puerto Rico	Mayaguaz		male	American Museum of Natural History
I. ramburii	11/20/25	Puerto Rico	Tortogueros lake, manati		orange female	American Museum of Natural History
I. ramburii	3/4/14	Puerto Rico	Arecibo		olive female	American Museum of Natural History
I. ramburii	7/27/14	Puerto Rico	Coamo Springs		male	American Museum of Natural History
I. ramburii	7/10/14	Puerto Rico	San Juan		male	American Museum of Natural History
I. ramburii	5/29/15	Puerto Rico	Caguaso		male	American Museum of Natural History
I. ramburii	12/31/14	Puerto Rico			male	American Museum of Natural History
I. ramburii	7/10/14	Puerto Rico	San Juan		male	American Museum of Natural History
I. ramburii	12/31/14	Puerto Rico			male	American Museum of Natural History
I. ramburii	1/8/14	Puerto Rico	San Turce		male	American Museum of Natural History
I. ramburii	11/20/25	Puerto Rico	Tortogueros lake, manati		male	American Museum of Natural History
I. ramburii	5/29/15	Puerto Rico	Caguas		olive female	American Museum of Natural History
I. ramburii	7/12/14	Puerto Rico	San Juan		olive female	American Museum of Natural History
I. ramburii	7/10/14	Puerto Rico	San Juan		olive female	American Museum of Natural History
I. ramburii	1/5/15	Puerto Rico	Jayuya		mimetic female	American Museum of Natural History
I. ramburii	6/6/15	Puerto Rico	Coamo Springs		male	American Museum of Natural History
I. ramburii	7/31/14	Puerto Rico			male	American Museum of Natural History
I. ramburii	7/10/14	Puerto Rico	San Juan		male	American Museum of Natural History
I. ramburii	11/10/25	Puerto Rico	Aibonito		male	American Museum of Natural History
I. ramburii	7/10/14	Puerto Rico	San Juan		male	American Museum of Natural History
I. ramburii	5/29/15	Puerto Rico	Caguas		olive female	American Museum of Natural History
I. ramburii	4/14/09	Puerto Rico	San Juan		male	American Museum of Natural History
I. ramburii	11/7/23	Puerto Rico	Mameres		male	American Museum of Natural History
I. ramburii	3/4/25	St. Croix			male	American Museum of Natural History

Table 5.S1, cont.

I. ramburii	4/7/25	St. Croix		male	American Museum of Natural History
I. ramburii	4/5/25	St. Croix		male	American Museum of Natural History
I. ramburii	4/6/35	St. Croix		male	American Museum of Natural History
I. ramburii	4/7/25	St. Croix		orange female	American Museum of Natural History
I. ramburii	6/4/17	St. Croix		orange female	American Museum of Natural History
I. ramburii	4/5/25	St. Croix		male	American Museum of Natural History
I. ramburii	3/7/25	St. Croix		orange female	American Museum of Natural History
I. ramburii	4/7/25	St. Croix		mimetic female	American Museum of Natural History
I. ramburii	3/4/25	St. Croix		male	American Museum of Natural History
I. ramburii	4/5/25	St. Croix		male	American Museum of Natural History
I. ramburii	3/1/25	St. Croix		male	American Museum of Natural History
I. ramburii	3/4/25	St. Croix		male	American Museum of Natural History
I. ramburii	11/5/25	St. John		male	American Museum of Natural History
I. ramburii	3/9/25	St. John		male	American Museum of Natural History
I. ramburii	3/8/25	St. John		male	American Museum of Natural History
I. ramburii	3/6/25	St. John		male	American Museum of Natural History
I. ramburii	3/5/25	St. John		male	American Museum of Natural History
I. ramburii	11/25/25	St. Thomas	St. Thomas	broken	American Museum of Natural History
I. ramburii	11/25/25	St. Thomas		male	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas		male	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas		male	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas		male	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas	-	orange female	American Museum of Natural History
I. ramburii	3/23/25	St. Thomas		orange female	American Museum of Natural History
I. ramburii	11/28/25	St. Thomas		orange female	American Museum of Natural History
I. ramburii	11/24/25	St. Thomas		orange female	American Museum of Natural History
I. ramburii	11/22/25	St. Thomas		male	American Museum of Natural History

Table 5.S1, cont.

I. ramburii	11/27/25	St. Thomas			bluish male	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas			orange female	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas			mimetic female	American Museum of Natural History
I. ramburii	11/23/25	St. Thomas	St. Thomas		mimetic female	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas			olive female	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas			mimetic female	American Museum of Natural History
I. ramburii	11/27/25	St. Thomas			male	American Museum of Natural History
I. ramburii	11/26/25	St. Thomas			mimetic female	American Museum of Natural History
I. ramburii	10/1/06	Tenessee	Henry Co., Paris Landing State Park	Connors	male	Gainesville
I. ramburii	10/1/06	Tenessee	Hamilton Co., Harrison Bay State Park	Connors	mimetic female	Gainesville
I. posita	8/1/01	Tenessee	Blout County, GSMNP, Cades Cove	Cook	female	Cook's private collection
I. ramburii	8/1/91	Texas	Del Rio, US-90 at San Filipe Creek Park	Mauffray	orange female	Gainesville
I. ramburii	6/22/42	Texas	10 miles south of Kerrville, TX	E.S. Ross	male	California Academy of Sciences*
I. ramburii	6/22/42	Texas	10 miles south of Kerrville, TX	E.S. Ross	orange female	California Academy of Sciences*
I. ramburii	8/29/42	Texas	Brownsville, TX	E.S. Ross	mimetic female	California Academy of Sciences*
I. ramburii	8/29/42	Texas	Brownsville, TX	E.S. Ross	male	California Academy of Sciences*
I. ramburii	8/29/42	Texas	Brownsville, TX	E.S. Ross	male	California Academy of Sciences*
I. ramburii	9/29/42	Texas	Brownsville,TX	E.S. Ross	male	California Academy of Sciences*
I. ramburii	9/28/42	Texas	Corpus Cristi, TX	E.S. Ross	male	California Academy of Sciences*
I. ramburii	9/29/42	Texas	Brownsville, TX	E.S. Ross	olive female	California Academy of Sciences*
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	orange female	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	mimetic	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	male	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	mimetic female	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	male	Gainesville

Table 5.S1, cont.

I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	male	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	orange female	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	male	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	male	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	male	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	olive female	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	olive female	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	olive female	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	olive female	Gainesville
I. ramburii	5/19/09	Trinidad	Edge of Caroni swamp Cacandee Village	Charles T. Collins	mimetic female	Gainesville
I. ramburii	8/1/86	Trinidad	Edge of Caroni swamp Cacandee Village	sw dunkle	male	Gainesville
I. ramburii	9/1/86	Trinidad	Edge of Caroni swamp Cacandee Village	sw dunkle	orange female	Gainesville
I. ramburii	2/11/53	Turks and Caicos	South Caicos		male	American Museum of Natural History
I. ramburii	2/11/53	Turks and Caicos	South Caicos		olive female	American Museum of Natural History
I. ramburii	2/11/53	Turks and Caicos	South Caicos		male	American Museum of Natural History
I. ramburii	4/1/94	unknown	Ft. clayton C.Z.	Frick	male	California Academy of Sciences*
I. ramburii	3/1/86	Venezuela	44k south of calabozo	miller and stange	male	Gainesville
I. ramburii	9/1/80	Venezuela	senare	westfall	male	Gainesville
I. ramburii	10/1/80	Venezuela	senare	westfall	orange female	Gainesville
I. ramburii	10/1/80	Venezuela	punta palmita, Lake Valencia	westfall	olive female	Gainesville
I. ramburii	9/1/80	Venezuela	lake in park sauta	westfall	olive female	Gainesville
I. ramburii	10/1/80	Venezuela	lake in park sauta	westfall	male	Gainesville
I. ramburii	9/1/80	Venezuela	senare	westfall	male	Gainesville

Table 5.S1, cont.

I. ramburii	10/1/80	Venezuela	punta palmita, Lake Valencia	westfall	male	Gainesville
I. ramburii	10/1/80	Venezuela	punta palmita, Lake Valencia	westfall	mimetic female	Gainesville
I. ramburii	10/1/80	Venezuela	punta palmita, Lake Valencia	westfall	mimetic female	Gainesville
I. ramburii	9/1/80	Venezuela	between el limon y caracas	westfall	mimetic female	Gainesville
I. ramburii	9/1/80	Venezuela	between el limon y caracas	westfall	male	Gainesville
I. ramburii	7/1/89	Venezuela	lagunillas sw of merida	donnelly	olive female	Gainesville
I. ramburii	7/1/89	Venezuela	lagunillas sw of merida	donnelly	orange female	Gainesville
I. ramburii	7/1/89	Venezuela	lagunillas sw of merida	donnelly	male	Gainesville
I. ramburii	7/1/89	Venezuela	lagunillas sw of merida	donnelly	male	Gainesville

Table 5.S2 Morph frequencies from *Ischnura ramburii* field sampling

adultandrofreq	summarycode	state	habitat	watershed	annual_prec	wettest.precipitation	ann_mean_temp	altitude	min_temp	driest_prec	pop	island	year	month	searchtime	males	females	oranges	olivebrs	blues	greens	density	sexratio	androfreq	bluewheel	greenwheel	orangewheel	olivebrownwheel	mean_egg_count
0.5	KAL08	HI	stream	south	960	130	242	31	178	37	KAL	Oahu	2008	7.5	240	19	13	6	6	2	6	0.13	0.59	0.4	0	0	0	0	NA
0.37	KAL09	HI	stream	south	960	130	242	31	178	37	KAL	Oahu	2009	6.5	285	58	25	3	7	10	4	0.29	0.7	0.58	0	2	0	3	230
0.4	MPA08	HI	stream	south	794	118	244	7	179	26	MPA	Oahu	2008	7.5	720	33	19	11	9	4	6	0.07	0.63	0.33	0	1	1	5	NA
0.5	MPA09	HI	stream	south	794	118	244	7	179	26	MPA	Oahu	2009	6.5	195	56	40	15	19	11	19	0.49	0.58	0.54	0	9	2	17	213
0.56	SLP09	HI	lake	south	911	128	242	19	178	31	SLP	Oahu	2009	6.5	120	42	28	3	8	7	10	0.58	0.6	0.61	0	5	1	6	239
0.6	WAP09	HI	pond	north	1948	254	239	70	179	99	WAP	Oahu	2009	6.5	400	51	33	7	4	14	6	0.21	0.61	0.65	0	1	0	3	255
0.5	WBP08	HI	lagoon	north	1030	159	234	53	170	32	WBP	Oahu	2008	7.5	120	16	5	5	7	1	7	0.18	0.76	0.4	0	3	0	1	NA
	WAP10	HI	pond	north	1948	254	239	70	179	99	WAP	Oahu	2010	7	180	36	22	13	0	9	0	0.32	0.62	0.41	0	0	0	0	NA
0.93	SLP10	HI	lake		911	128	242	19	178	31	SLP	Oahu	2010	7	60	33	23	4	1	4	14	0.93	0.59	0.78	0	3	1	0	366
0.53	MPA10	HI	stream		794	118	244	7	179	26	MPA	Oahu	2010	7	70	22	36	8	7	13	8	0.83	0.7	0.58	0	5	0	6	305
0.08	ANA08	HI	puddle	east	1208	174	237	6	174	39	ANA	Kauai	2008	8	330	14	14	8	11	0	1	0.08	0.5	0.05	0	1	0	7	230
0.14	CCP09	HI	pond	east	1281	188	238	7	175	51	CCP	Kauai	2009	8	170	44	40	18	18	1	3	0.49	0.52	0.1	0	0	0	6	238
0.4	HAN09	HI	pond	north	2107	247	236	6	173	88	HAN	Kauai	2009	8	120	30	13	5	3	3	2	0.36	0.7	0.38	0	0	0	2	145
0.43	KGP09	HI	pond	east	1821	229	231	97	167	82	KGP	Kauai	2009	8	60	16	13	5	4	1	3	0.48	0.55	0.31	0	1	0	1	194
0.29	KLG09	HI	pond	east	1119	153	237	35	174	46	KLG	Kauai	2009	8	67	35	22	13	5	2	2	0.48	0.61	0.18	0	1	0	5	265
0.16	MEN09	HI	pond	east	1104	149	238	5	176	47	MEN	Kauai	2009	8	240	49	44	22	16	3	3	0.39	0.53	0.14	0	1	0	5	187
0.33	NAW08	HI	lagoon	east	1092	151	237	25	175	44	NAW	Kauai	2008	7.5	220	15	4	3	6	1	3	0.09	0.79	0.31	0	0	0	3	NA
0.25	NAW09	HI	lagoon	east	1092	151	237	25	175	44	NAW	Kauai	2009	8	80	12	15	8	3	3	1	0.34	0.44	0.27	0	0	0	0	NA
0.08	NBG09	HI	lagoon	south	1192	151	236	50	172	59	NBG	Kauai	2009	8	300	29	16	3	12	0	1	0.15	0.64	0.06	0	0	1	11	249

Table 5.S2, cont.

0.14	POP09	HI	pond	south	1060	142	238	17	174	49	POP	Kauai	2009	8	60	10	9	2	6	0	1	0.32	0.53	0.11	0	1	0	4	323
0.39	PRV09	HI	pond	north	2442	289	233	59	170	110	PRV	Kauai	2009	8	320	122	68	21	25	6	16	0.59	0.64	0.32	0	5	0	15	238
0.5	RSP08	HI	stream	east	1688	202	233	88	170	90	RSP	Kauai	2008	7.5	120	19	5	3	1	1	1	0.2	0.79	0.33	0	0	0	0	NA
0.13	SPH09	HI	pond	south	1082	145	237	29	173	51	SPH	Kauai	2009	8	160	65	42	17	21	1	3	0.67	0.61	0.1	0	0	2	15	213
0.29	TAR08	HI	pond	north	2256	261	236	8	173	95	TAR	Kauai	2008	7.5	400	26	16	10	10	6	4	0.11	0.62	0.33	1	1	0	4	NA
1	TAR09	HI	pond	north	2256	261	236	8	173	95	TAR	Kauai	2009	8	60	9	6	4	0	0	2	0.25	0.6	0.33	0	0	0	0	NA
0.14	WCR08	HI	pond	south	646	123	238	6	173	13	WCR	Kauai	2008	8	120	27	12	15	6	4	1	0.33	0.69	0.19	1	1	0	4	NA
0.05	WCR09	HI	pond	south	646	123	238	6	173	13	WCR	Kauai	2009	8	270	54	31	12	18	0	1	0.32	0.64	0.03	0	1	1	14	276
0.47	WTF09	HI	pond	north	2032	240	236	11	173	85	WTF	Kauai	2009	8	160	30	32	12	10	1	9	0.39	0.48	0.31	0	3	1	8	258
0.4	PRV10	HI	pond		2442	289	233	59	170	110	PRV	Kauai	2010	7	580	325	196	71	68	21	45	0.9	0.9	0.32	1	18	2	39	236
0.2	KBP10	HI	pond		1782	215	229	162	164	94	KBP	Kauai	2010	7	90	25	12	7	4	0	1	0.4	0.68	0.08	0	0	0	0	275
0.12	WCR10	HI	pond		646	123	238	6	173	13	WCR	Kauai	2010	8	360	51	28	11	15	0	2	0.22	0.65	0.07	0	2	4	8	169
0.15	KLG10	HI	pond		1119	153	237	35	174	46	KLG	Kauai	2010	8	315	58	32	17	11	2	2	0.29	0.64	0.13	0	1	1	8	261
0.2	CCP10	HI	pond		1281	188	238	7	175	51	CCP	Kauai	2010	8	135	24	14	4	9	0	1	0.28	0.63	0.07	0	1	0	5	208
0.32	TAR10	HI	pond		2256	261	236	8	173	95	TAR	Kauai	2010	8	1210	208	180	61	70	16	33	0.32	0.54	0.27	0	16	10	50	226
0.45	POL09	HI	lagoon	north	1199	157	237	2	180	46	POL	Hawaii	2009	7	140	29	26	3	11	3	9	0.39	0.53	0.46	0	4	0	6	145

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