Original Article

Multiple routes to reduced interspecific territorial fighting in *Hetaerina* damselflies

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Interspecific territoriality may be adaptive if territories contain depletable resources that are valuable to both species, but it can also arise as a maladaptive by-product of intraspecific territoriality. In the latter scenario, sympatric species ought to diverge in ways that reduce interspecific fighting. We studied 4 *Hetaerina* damselfly species that can be found in sympatry in North America. Prior work showed that sympatric populations have diverged from each other in wing coloration and competitor recognition in 2 of the 4 sympatric species pairs (*H. titia*/*H. occisa*, *H. titia*/*H. americana*). Here, we show that sympatric populations of these 2 species pairs overlap completely in habitat use, and yet, interspecific territorial fights occur much less frequently than intraspecific fights. Experimentally manipulating the wing coloration of male *H. occisa* and *H. americana* to more closely resemble *H. titia* increased the rate of interspecific fights, which provides direct evidence that divergence in wing coloration is partly responsible for the low rate of interspecific fights. We found that interspecific fighting is also reduced in the other 2 species pairs (*H. occisa*/ *H. cruentata*, *H. americana*/*H. cruentata*), even though prior work showed that heterospecific territory intruders are attacked just as aggressively as conspecific territory intruders. In these cases, however, the sympatric species differ sufficiently in habitat use to reduce the interspecific encounter rate and thereby account for the reduced rate of interspecific fighting. Thus, interspecific fighting is reduced relative to intraspecific fighting in all 4 species pairs, albeit through different mechanisms. *Key words:* competitor recognition, heterospecific aggression, interspecific fighting, interspecific territoriality, species recognition. *[Behav Ecol 22:527–534 (2011)]*

INTRODUCTION

In their classic review of interspecific territoriality in birds, Orians and Willson (1964) described several possible outcomes of secondary contact between formerly allopatric territorial species. In one scenario, the newly sympatric species initially respond aggressively to each other and defend non-overlapping territories, but interspecific aggression is selected against because the species overlap so little in resource use that the costs of defending interspecific territories outweigh the benefits. Orians and Willson predicted that this would lead to divergence in the traits used to identify conspecifics until the species no longer respond aggressively to each other. They did not consider this character displacement scenario further, however, because they were primarily concerned with cases in which interspecific territoriality is adaptive. For the most part, researchers studying interspecific territoriality have continued to focus on cases in which it seems adaptive, while those interested in character displacement have focused on the classic mechanisms described by Brown and Wilson (1956; but see Cody 1969, 1973; Gill 1974; Murray 1981; Adams 2004; Tynkkynen et al. 2005; Peiman and Robinson 2007; Grether et al. 2009). Brown and Wilson (1956) alluded to the possibility that interspecific fighting could cause sympatric character shifts but only to clarify that they did not intend their theory of character displacement to encompass such shifts.

Recently, it has become clear that interspecific fighting is common among closely related or phenotypically similar species (Schultz and Switzer 2001; Dijkstra et al. 2005, 2006; Tynkkynen et al. 2006; Pauers et al. 2008; Peiman and Robinson 2010). Moreover, interspecific fighting is not restricted to species that compete with each other for limited resources (Grether et al. 2009; Ord and Stamps 2009; Peiman and Robinson 2010). Grether et al. (2009) modeled the character displacement scenario described by Orians and Willson (1964) and showed that 2 species that initially treat each other as competitors but are not in competition for a limiting resource can diverge in agonistic signals and competitor recognition functions until they no longer interact aggressively. Conversely, species that do not initially recognize each other as competitors but are in competition for a limiting resource can converge in agonistic signals and competitor recognition functions until they treat each other as competitors, as suggested by Cody (1969, 1973). Several putative examples of both of these forms of "agonistic" character displacement have been reported (Tobias and Seddon 2009; Anderson and Grether 2010a), but in general, the evolutionary effects of interspecific fighting remain understudied (Grether et al. 2009; Peiman and Robinson 2010).

In this paper, we further investigate recently reported cases of divergent agonistic character displacement in *Hetaerina* damselflies (Anderson and Grether 2010a, 2010b). We explain the specific goals of this paper after introducing the study system and the evidence for character displacement.

Male *Hetaerina* damselflies form leks along rivers where they defend small non-resource–based mating territories. Both mating and territorial behavior typically occurs between the hours of 10:00 and 17:00; outside this period, males are

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nonterritorial and readily share perches while roosting or hunting for aerial prey (Weichsel 1987; Grether 1996; Grether and Grey 1996; Grether and Switzer 2000). Unlike in *Calopteryx* damselflies, males do not defend access to oviposition sites (Alcock 1987; Cordoba-Aguilar et al. 2007). A *Hetaerina* territory is merely a defended space within which the resident perches and has priority of access to arriving females (Weichsel 1987; Grether 1996). Although territories are commonly the sites of mate acquisition, mating pairs usually leave the male's territory to search for a suitable site to oviposit. Territorial contests are common, conspicuous, and energetically expensive and have important fitness consequences (Grether 1996; Contreras-Garduno et al. 2006; Serrano-Meneses et al. 2007). Given that mates are a species-specific resource, there would appear to be no benefit to attacking heterospecific males. Nevertheless, interspecific territorial fights do occur (Johnson 1963), and some sympatric *Hetaerina* species defend exclusive nonoverlapping and interspecific territories (this paper).

Our research has focused on 4 sympatric species pairs: *H. americana*/*H. titia*, *H. occisa*/*H. titia*, *H. americana*/ *H. cruentata*, and *H.occisa*/*H. cruentata*. Males of every species in this genus have red spots at the base of the forewings (hence the common name, rubyspot damselflies). Females lack red wing pigmentation and in general are more cryptic in coloration than males (Figure 1). Hence, the red wing spots of males are classic secondary sexual characters (Darwin 1871). *Hetaerina titia* is the only species in the genus that has substantial amounts of black wing pigmentation. Males of other 3 species have red spots at the base of all 4 wings, and the rest of the wing area is mostly transparent (Garrison 1990; Westfall and May 1996). The black wing spots of male *H. titia* are highly variable and range in size from a small portion of the wing base (similar in size to sympatric congeners) to black areas covering the entire hind wing and most of the forewing (Johnson 1963; Garrison 1990; Anderson and Grether 2010b; Figure 1). Thus, *H. americana*/*H. titia* and *H. occisa*/*H. titia* represent pairs of species with dissimilar wing coloration, whereas *H. americana*/*H. cruentata* and *H. occisa*/*H. cruentata* represent pairs of species with similar wing coloration.

In simulated territory intrusion tests, in which live tethered males were literally flown into the territories of other males, residents responded more aggressively to conspecific intruders than to heterospecific intruders in the species pairs with dissimilar wing coloration but not in the species pairs with similar wing coloration (Anderson and Grether 2010a). That wing coloration is at least partly responsible for these results was demonstrated by adding black coloration to the wings of *H. occisa* and *H. americana* intruders. Conspecific residents attacked blackened intruders at reduced rates compared with sham-manipulated control intruders, whereas *H. titia* residents attacked blackened intruders at increased rates compared with sham-manipulated control intruders. At allopatric sites, where *H. titia* is not present, the same color manipulation had no effect on the responses of residents to conspecific intruders, which suggests that competitor recognition has diverged in sympatry (Anderson and Grether 2010a).

Based solely on the responses of males to simulated territory intruders, we would expect interspecific fighting to be reduced relative to intraspecific fighting in the species pairs with dissimilar coloration, whereas no such reduction in interspecific fighting would be expected in the species pairs with similar coloration. Territory intrusion tests only show what happens when animals actually encounter each other, however. Selection against interspecific fighting could also cause sympatric species to diverge in ways that reduce the rate of interspecific territorial encounters (e.g., divergence in habitat preferences). Here, we examine how naturally occurring rates of fighting and territory spacing vary among the 4 species pairs. To evaluate species differences in microhabitat use, we compare the percent canopy cover and current speed of territories. These microhabitat variables were selected because light and current speed have previously been shown to influence habitat use in *Hetaerina* (Johnson 1962, 1966; Weichsel 1987; Grether 1996; Grether and Switzer 2000). Lastly, we test for effects of wing coloration on rates of interspecific fighting in free-flying males using both observational and experimental approaches.

MATERIALS AND METHODS

Localities and data collection

The observations reported here were carried out in July 2005; June–July 2006; June–July 2007; and in April, May, and August 2008 at 6 sites in Texas and Mexico (site name abbreviations and GPS coordinates are given): Castroville, Texas (CV: 29.34 N, 98.88 W); El Limon, Mexico (EL: 21.37 N, 104.62 W); Armeria, Mexico (AR: 18.96 N, 103.95 W); southeast of La Tinaja, Mexico (OT: 18.69 N, 96.39 W); La Palma, Mexico (PA: 18.56 N, 95.07 W); and Tebanca, Mexico (CT: 18.37 N, 95.00 W). At 3 sites, we performed replicate observations: AR (July 2005, April 2008), CV (April 2008, August 2008), and OT (June 2006, June 2007). Data from repeat visits to the same site were not pooled because expected values for rates of intra- and interspecific fights under the null hypothesis depend on relative species abundances, which varied somewhat between visits.
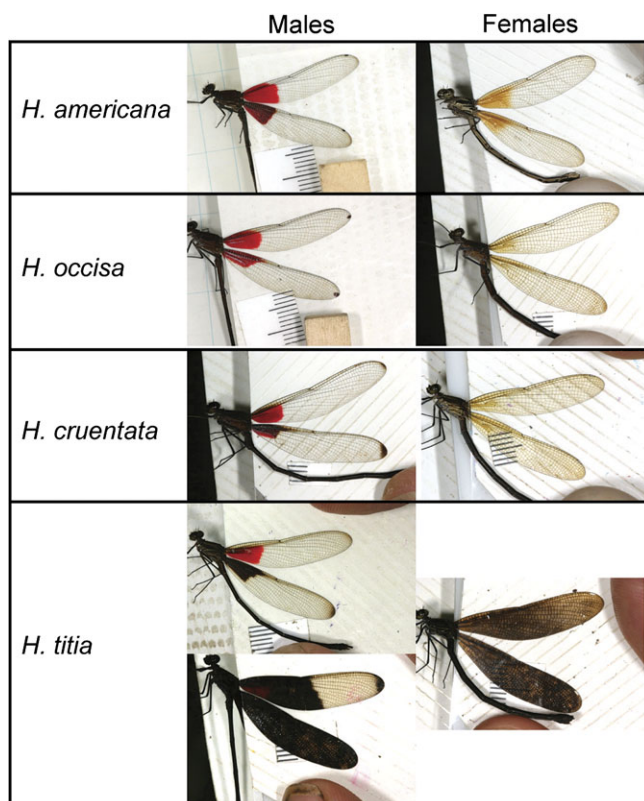
Figure 1
Wing color variation in studied *Hetaerina* species. One male and one female are shown for all species, except 2 males are shown for *H. titia* to demonstrate the extensive variation in wing coloration. Images not to scale.

Study transects (50–100 m) were established along riverbanks. Damselflies were captured with aerial nets, photographed, and individually marked with a unique combination of 3 colored marks on the abdomen using paint pens (Marvy Decocolor Paint Marker; Fine Point, Uchida of America, Torrance, CA). Wings were photographed with a digital camera (Canon 10D; Canon USA, Inc., Lake Success, NY) equipped with a 100 mm macro lens and macro flash attachment (Canon MT-24EX). Behavioral data collection began after at least 7 h were devoted to marking damselflies within the study transect, by which time >80% of the males were marked. During a behavioral census, we recorded each individual's perch location relative to the transect line to the nearest 0.1 m and all observed fights, regardless of whether the individuals were marked. Some marked males were recorded in multiple fights. Repeated fights between the same 2 marked males were reduced to a single observation; otherwise, we treated multiple observations of the same male fighting with different marked or unmarked individuals as independent observations. Transects were censused hourly between 10:00 and 17:00 for at least 3 consecutive days (range: 3–8 days). With one exception, all observations were recorded on consecutive days. Inclement weather interrupted one census day at the Castroville site. For the purposes of territory mapping (see below), we treated days adjacent to the inclement weather as consecutive.

To estimate microhabitat use of each species, along each transect, we estimated canopy cover at every 10 m using a concave spherical densiometer (Model C Spherical Crown Densiometer; Forestry Suppliers, Jackson, MS), and stream current speed at every 2 m using a categorical scale based on the appearance of the water surface (0: still; 1:moving but flat; 2: shallow standing waves; 3: rippling/mixing; and 4: turbulent/white water). Canopy cover was estimated at the river's midpoint if the river was less than 10 m wide; for rivers with widths that exceeded 10 m, we estimated canopy cover at the river's midpoint and at 2 m from each riverbank. We interpolated these measurements to estimate percent canopy cover and current speed for each territory midpoint.

Measurements of total hind wing area and the extent of pigmentation were made using NIH Image software (US National Institutes of Health; available on the Internet at http://rsb.info.nih.gov/nih-image/). Wing spot size was measured as the proportion of total wing area covered with pigment.

Territoriality

Territorial males often displayed a high degree of perch site fidelity, but our threshold for classifying a male as holding a territory at a given site was that he was recorded within a 3 m radius for at least 2 consecutive days. Territory midpoints were determined by averaging the x–y locations of the same individual on a given day. Nearest-territorial-neighbor distances and territory "neighborhood" densities were calculated separately for each individual on each day of the study. For 2 territorial residents to occupy the same neighborhood, their territory midpoints could be no further than 4 m apart. This distance criterion was based on the observation that territorial males chase away intruding males that come within approximately 1–2 m of their perch (Raihani et al. 2008; Anderson CN and Grether GF, personal observation).

If interspecific territoriality is weaker than intraspecific territoriality, and species do not differ in habitat preferences, we would expect territorial neighborhoods to be biased toward heterospecifics. That is, we would expect a 4 m radius drawn around a given male's territory midpoint to include more heterospecific residents than conspecific residents, on average, after adjusting for species differences in density. Conversely, if interspecific territoriality is stronger than intraspecific territoriality or if the species differ in habitat preferences, then we would expect territorial neighborhoods to be biased toward conspecifics. Finally, if inter- and intraspecific territoriality are equal in strength, and the species do not differ in habitat preferences, we would expect no bias in either direction.

Wing color manipulation experiment

At a subset of sites, we manipulated the wing coloration of *H. americana* or *H. occisa* to resemble the wing coloration of *H. titia* and documented the effect on interspecific fights. This experiment was carried out not only at *H. americana*/*H. titia* and *H. occisa*/*H. titia* sites but also at *H. americana*/*H. cruentata* and *H. occisa*/*H. cruentata* sites, to establish whether the response to the treatment was predicted by species composition. Previously marked *H. americana* and *H. occisa* males were recaptured and then assigned 1 of 3 treatments: 1) blackened—hind wings fully painted with a black marker (Prismacolor PM-2; Sanford L.P., Oak Brook, IL); 2) sham control—hind wings painted with a colorless marker (Prismacolor PM-121); or 3) unmanipulated control. All males were released at the location of capture immediately after treatment. The black marker closely matches the color of *H. titia* wings both to human eyes and as assessed with reflectance spectrometry. Treatments were assigned at random with respect to the identity of the male but in alternating order to ensure that half received the blackened treatment, one quarter received the sham control treatment, and one quarter received the unmanipulated control treatment. After the last male was added to the study, hourly censusing continued for 3–4 consecutive days.

Data analysis—fighting

To examine whether the observed frequencies of intra- and interspecific fights differed significantly from null expectations, we used a chi-squared test. Expected frequencies were generated from a binomial expansion of the proportion of marked individuals of each species. To test the prediction that wing coloration influences rates of interspecific fighting, we used a randomization test (sensu Whitlock and Schluter 2009) comparing the mean wing spot size of individually marked *H. titia* observed in interspecific fights with a null distribution of means from 10 000 simulated data sets obtained by resampling (without replacement) from a pool of all marked *H. titia* from the site. We restricted this analysis to sites where at least 5 marked *H. titia* were observed in interspecific fights. Randomization tests were performed in R 2.8 (R Foundation for Statistical Computing, Vienna, Austria).

In the wing color manipulation experiment, we predicted that blackening *H. americana* or *H. occisa* wings would increase the frequency of fights with *H. titia* but have little or no effect on the frequency of fights with *H. cruentata*. Chi-square tests were used to compare the frequencies of interspecific fights involving experimentally blackened or control males. Control groups were pooled after verifying that the sham treatment had no significant effect on fighting rates compared with the unmanipulated control (exact test: $P = 0.866$). Expected frequencies for interspecific fights were generated from the proportion of individuals that received each treatment. Chi-squared analyses were performed in StatXact 8 (Cytel Software Corporation, Cambridge, MA). When expected values were below 5, P values were calculated by Monte Carlo simulation.

Data analysis—spacing and microhabitat use

A generalized linear mixed model (GLMM) was constructed to compare the observed proportion of heterospecific neighbors with an expected proportion of heterospecific neighbors for each species at each site separately. Expected proportions were generated from each species' relative abundance on
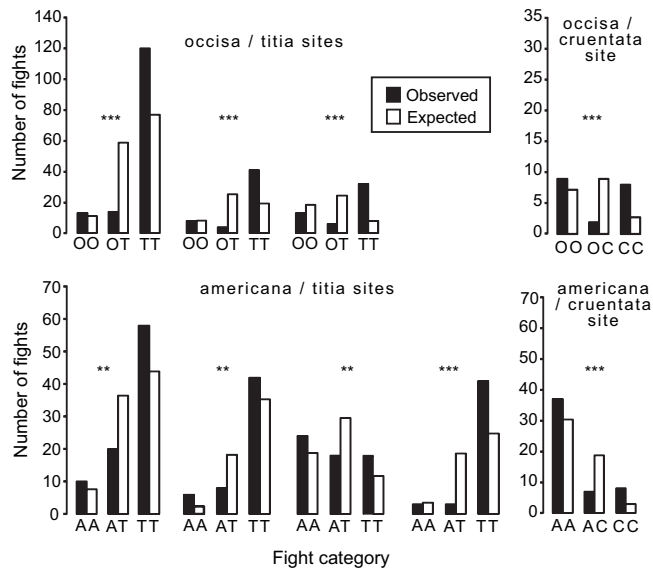
each territorial day. Each GLMM was fit with a binomial error structure and logit link function, with the proportion of heterospecific neighbors as the response variable and a categorical explanatory term that coded observed and expected frequencies of neighbors. All GLMMs used the cluster command in Stata 10 (StataCorp LP, College Station, TX) to adjust the standard error for repeated measurements on the same territory holders. To compare conspecific and heterospecific nearest neighbor distances, we used the nearest neighbor distance on each territorial day as the response variable in a regression model with a categorical explanatory term coding heterospecific versus conspecific status. We used the cluster command in Stata to adjust the standard error for repeated measurements on the same territory holder. Analyses treating each of 2 sympatric species as the focal species at a given site are not independent of each other. Therefore, to avoid redundancy, here, we only present results from analyses where *H. titia* or *H. cruentata* was treated as the focal species. This choice of perspective facilitates comparisons of species pairs with similar versus dissimilar coloration. Results from analyses treating *H. americana* or *H. occisa* as the focal species are presented in the online supplement.

Wilcoxon rank-sum tests were used to test for species differences in territory canopy cover and current speed. GLMM, regression, and Wilcoxon rank-sum tests were performed in Stata 10. Unless otherwise stated, all reported P values are two tailed.

RESULTS

Fighting

The frequency of interspecific fights was lower than random expectations at all *H. occisa* and *H. americana* sites, regardless of whether the sympatric congener was *H. titia* or *H. cruentata*

(Figure 2; OT in 2006: chi-square statistic $[\chi^2] = 58.55$, 3 degrees of freedom [df], $P < 0.001$; OT in 2007: $\chi^2 = 42.00$, 3 df, $P < 0.001$; PA: $\chi^2 = 88.18$, 3 df, $P < 0.001$; AR in 2005: $\chi^2 = 12.74$, 3 df, $P = 0.0017$; AR in 2008: $\chi^2 = 12.63$, 3 df, $P = 0.0018$; CV in April 2008: $\chi^2 = 9.4$, 3 df, $P = 0.0089$; CV in August 2008: $\chi^2 = 23.9$, 3 df, $P < 0.001$; CT: $\chi^2 = 15.78$, 3 df, $P < 0.001$; and EL: $\chi^2 = 17.9$, 3 df, $P < 0.001$). As predicted, at *H. titia* sites, blackening the wings of male *H. occisa* and *H. americana* caused them to get into more interspecific fights than control males (Figure 3; PA: $\chi^2 = 6.3$, 2 df, $P = 0.012$; OT in 2007: $\chi^2 = 19.6$, 2 df, $P < 0.001$; and AR in 2008: $\chi^2 = 7.9$, 2 df, $P = 0.005$). At *H. cruentata* sites, blackening the wings of *H. occisa* had no effect on the rate of interspecific fights (Figure 3; CT: $\chi^2 = 0.00054$, 2 df, $P = 0.98$;), whereas blackening the wings of *H. americana* decreased the rate of interspecific fights (Figure 3; EL: $\chi^2 = 8.48$, 2 df, $P = 0.004$).

As predicted, *H. titia* observed in fights with *H. occisa* had smaller black wing spots than the null expectation (Figure 4: OT: $P_{\text{one tailed}} = 0.0046$; PA: $P_{\text{one tailed}} = 0.028$). This was not the case for *H. titia* observed in fights with *H. americana* (Figure 4: AR in 2005, $P_{\text{one tailed}} = 0.26$; AR in 2008, $P_{\text{one tailed}} = 0.83$; and CV in April 2008, $P_{\text{one tailed}} = 0.84$), but the power to detect effects of *H. titia* wing spot size was lower at *H. americana* sites than at *H. occisa* sites because *H. titia* wing spot size was much less variable at *H. americana* sites (mean 95% confidence interval [CI]: 5.97%) than at *H. occisa* sites (mean 95% CI: 32.6%; Figure 3). In short, *H. titia* wing spots were not sufficiently variable at *H. americana* sites to perform a strong test of this prediction.

Spacing and microhabitat use

Hetaerina titia territorial neighborhoods were always biased toward heterospecifics at *H. occisa* sites (Table 1), which implies that interspecific territoriality is weaker than intraspecific
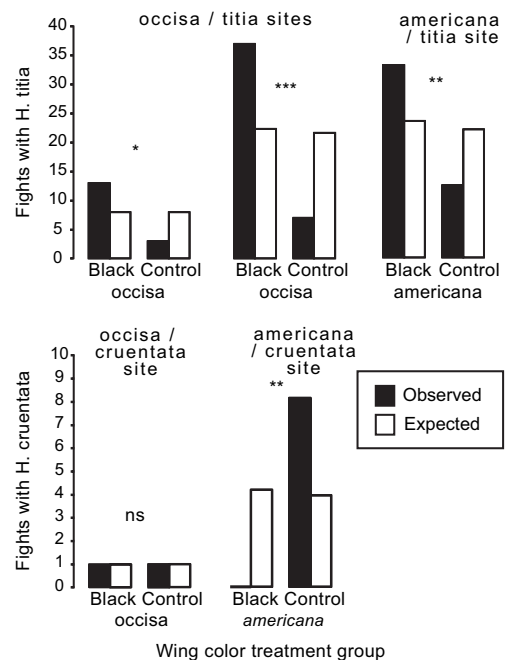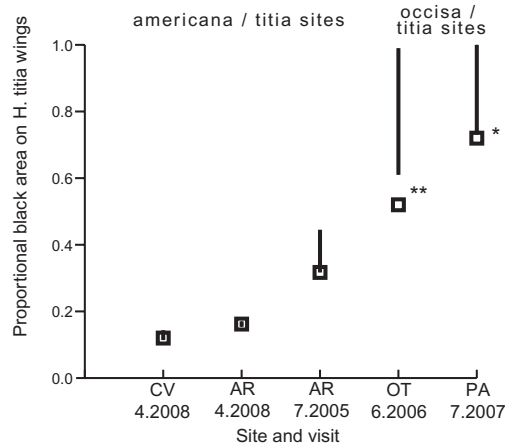

Figure 2
Observed and expected frequencies of intra- and interspecific fights. Codes: OO, *occisa* versus *occisa*; OT, *occisa* versus *titia*; TT, *titia* versus *titia*; AA, *americana* versus *americana*; AT, *americana* versus *titia*; AC, *americana* versus *cruentata*; CC, cruentata versus cruentata; and OC, *occisa* versus *cruentata*. Site and visit, Top Row: OT, 6.2006; OT, 7.2007; PA, 7.2007; and CT, 7.2006; Bottom Row: AR, 7.2005; AR, 4.2008; CV, 4.2008; CV 8.2008; and EL, 5.2008. In all instances, observed interspecific fights are less frequent than null expectations. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Figure 3
Observed and expected number of interspecific fights for blackened and control males in the wing color manipulation experiment. Site and visit, Top Row: PA, 7.2007; OT, 6.2007; and AR, 4.2008; Bottom Row: CT, 7.2006; EL, 4.2008. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Figure 4
Average hind wing spot of *Hetaerina titia* observed in interspecific fights (squares) compared with a 95% CI of *H. titia* hind wing spot size in the local population (vertical lines). Asterisks correspond to one-tailed *P* values based on a randomization test. *$P < 0.05$, **$P < 0.01$, all others NS, not significant.

territoriality in this species pair. At *H. americana* sites, *H. titia* territorial neighborhoods were strongly biased toward heterospecifics at one site in 1 year, but no bias was observed at the same site in a different year or at the other site where these 2 species occur (Table 1). *Hetaerina cruentata* neighborhoods were always biased toward conspecifics, whether the sympatric congener was *H. americana* or *H. occisa* (Table 1), which is consistent with our evidence for habitat partitioning (see below). Analyses carried out with *H. americana* or *H. occisa* as the focal species yielded similar results (see Supplementary Material, Supplementary Table S1).

Compared with *H. titia* residents with conspecific nearest neighbors, *H. titia* residents with *H. americana* nearest neighbors were significantly closer to their nearest neighbors at one site (CV in April 2008, August 2008; Table 2) but not at a second site (AR in 2005, 2008; Table 2). Likewise, compared with *H. titia* residents with conspecific nearest neighbors, *H. titia* residents with *H. occisa* nearest neighbors were significantly closer to their nearest neighbors at one site in 1 year but not at the same site in a different year or at a second site (Table 2). *Hetaerina cruentata* nearest neighbor distance did not depend on whether the nearest neighbor was conspecific or heterospecific, regardless of whether the sympatric congener was *H. americana* or *H. occisa*. Analyses carried out with *H. americana* or *H. occisa* as the focal species yielded similar results (Supplementary Material, Supplementary Table S2).

Average percent canopy cover did not differ significantly between the territories of *H. titia* and the territories of either of its sympatric congeners (Table 3). *Hetaerina cruentata* territories differed in average percent canopy cover from those of its sympatric congeners, but the direction of the difference depended on the species pair. *Hetaerina cruentata* territories were shadier than *H. americana* territories but less shady than *H. occisa* territories (Table 3). *Hetaerina cruentata* occupied significantly shadier territories at the *H. americana* site than at the *H. occisa* site (Wilcoxon test, $z = 5.854$, $P < 0.0001$, $n = 71$).

Current speed did not differ significantly between the territories of *H. titia* and the territories of either of its sympatric congeners (Table 4). *Hetaerina cruentata* territories had more rapid flow than *H. occisa* territories but did not differ significantly from *H. americana* territories (Table 4).

DISCUSSION

In all 4 sympatric *Hetaerina* species pairs, interspecific fights occurred less frequently than expected based just on relative species densities. This reduction in interspecific interference is expected in species, like *Hetaerina*, that defend non-resource–based territories. In the species pairs with similar wing coloration (*H. americana/H. cruentata* and *H. occisa/H.cruentata*), the reduction in interspecific interference probably results from species differences in microhabitat use that reduce the rate of interspecific encounters relative to intraspecific encounters. Our evidence for this is that territory neighborhoods were biased toward conspecifics (Table 1, Supplementary Material, Supplementary Table S1), and the territories of sympatric species differed in mean canopy cover and/or current speed (Tables 3 and 4). Species-level differences in microhabitat use cannot entirely account for these results, however. Mean canopy cover was much greater on *H. cruentata* territories at site EL (47.7%) than at site CT (7.3%). For the sympatric congeners, however, the site differences in mean canopy cover went in the opposite direction (15.8% for *H. americana* at site EL vs. 25.9% for *H. occisa* at site CT). This shows that the *H. cruentata* results cannot be explained entirely by site differences in mean canopy cover. Sunny territories were available at EL, but they were occupied by *H. americana*. Likewise, shady territories were available at CT, but they were occupied by *H. occisa*. We cannot infer causal mechanisms from these results, but we can rule out the hypothesis that the site difference in habitat use by *H. cruentata* is solely due to site differences in shade availability. Comparable data from a wider range of sympatric and allopatric sites will be required to determine whether the direction of the species differences in shade use are predictable, and manipulative field experiments will be required to

Table 1

Observed and expected neighborhood compositions for territorial male *Hetaerina titia* and *H. cruentata*

Focal species	N	Sympatric congener	Site	Visit (month year)	Observed prop.of heterosp.neighbors	SE	Expected prop.of heterosp. neighbors	z	P
H. titia	28	*H. americana*	AR	July 2005	0.456	0.063	0.479	0.370	0.709
H. titia	70	*H. americana*	AR	April 2008	0.342	0.034	0.323	−0.550	0.586
H. titia	30	*H. americana*	CV	April 2008	0.588	0.051	0.578	−0.200	0.838
H. titia	20	*H. americana*	CV	August 2008	0.441	0.092	0.417	−0.260	0.797
H. titia	46	*H. occisa*	OT	June 2006	0.401	0.042	0.290	−3.410	0.001
H. titia	41	*H. occisa*	OT	June 2007	0.491	0.041	0.396	−2.430	0.015
H. titia	26	*H. occisa*	PA	July 2007	0.929	0.026	0.644	−4.910	<0.001
H. cruentata	24	*H. americana*	EL	May 2008	0.319	0.074	0.711	4.960	<0.001
H. cruentata	44	*H. occisa*	CT	July 2006	0.202	0.060	0.415	3.560	<0.001

See METHODS for key to Study sites and for description of neighborhood composition metrics. *P* values are based on GLMMs. SE, standard error.

Table 2

Conspecific and heterospecific nearest neighbor distances for territorial male *Hetaerina titia* and *H. cruentata*

Focal species	Sympatric congener	Site	Visit (month year)	Conspecific distance (meters)	SE	Heterospecific distance (meters)	SE	t	P
Hetaerina titia	*H. americana*	AR	July 2005	1.393(18)	0.232	1.944(14)	0.276	−1.960	0.060
H. titia	*H. americana*	AR	April 2008	1.498(55)	0.092	1.562(35)	0.140	−0.390	0.698
H. titia	*H. americana*	CV	April 2008	1.618(23)	0.188	1.174(23)	0.153	2.300	0.029
H. titia	*H. americana*	CV	August 2008	2.233(14)	0.182	1.411(9)	0.303	2.350	0.030
H. titia	*H. occisa*	OT	June 2006	1.562(36)	0.172	1.192(29)	0.134	2.050	0.047
H. titia	*H. occisa*	OT	June 2007	1.472(27)	0.189	1.043(34)	0.117	1.860	0.070
H. titia	*H. occisa*	PA	July 2007	1.175(3)	0.696	1.708(25)	0.212	−1.010	0.320
H. cruentata	*H. americana*	EL	May 2008	1.535(19)	0.178	1.682(11)	0.269	−0.560	0.578
H. cruentata	*H. occisa*	CT	July 2006	1.959(35)	0.140	2.228(17)	0.172	1.260	0.215

See METHODS for key to Study sites. Sample sizes are shown in parentheses. *P* values are based on regression models. SE, standard error.

determine whether the inferred shifts in habitat use are a product of past or current interspecific aggression. Data from a wider range of sites may additionally indicate whether regular dominance relationships are present among species pairs, which may influence whether one or both species diverge from allopatric habitat utilization patterns.

In the 2 species pairs with dissimilar wing coloration (*H. titia*/*H. americana* and *H. titia*/*H. occisa*), analyses of territory spacing revealed either no spatial bias or closer proximity to heterospecifics than to conspecifics (Tables 1 and 2, Supplementary Material, Supplementary Tables S1 and S2). No species differences in microhabitat use were detected in these species pairs (Tables 3 and 4). Instead, interspecific fighting appears to be reduced by divergence in wing coloration–based competitor recognition (Anderson and Grether 2010a). This hypothesis predicts that males whose wing coloration more closely resembles that of a sympatric congener will experience higher rates of interspecific fighting. We tested this prediction in 2 ways: first, by manipulating the wing coloration of male *H. occisa* and *H. americana* to more closely resemble *H. titia* and second, by taking advantage of the high level of natural variation in *H. titia* wing spot size at some sites. As predicted, male *H. occisa* and *H. americana* whose wings were painted black to resemble the wing coloration of *H. titia* were observed in more fights with *H. titia* than were control males (Figure 2). Likewise, *H. titia* that were observed in interspecific fights had smaller black wing spots, on average, than expected by chance (Figure 3). These findings are consistent with those of Johnson (1963) who reported that *H. titia* with small black wing spots were more often displaced from perch sites by *H. americana* than were *H. titia* with large black wing spots. Similar correlations between wing coloration and interspecific fighting have been reported in *Calopteryx* damselflies (Tynkkynen et al. 2004, 2006).

Thus, we found that interspecific fighting is reduced relative to intraspecific fighting in all 4 species pairs, albeit through different mechanisms and to different degrees. Whether the variability in outcomes is predictable, or at least explicable, is a question that would require data on more species and an explicitly phylogenetic (e.g., ancestral state reconstruction) approach. Perhaps some threshold level of pre-secondary contact divergence in wing coloration is required to enable evolution to take the route of character displacement in competitor recognition and wing coloration. Alternatively, or in addition, divergence in habitat preferences prior to secondary contact might reduce the frequency of interspecific fights sufficiently to eliminate selection for divergence in competitor recognition and coloration.

The highly variable wing coloration of *H. titia* is a prime candidate for agonistic character displacement. Because we have not found *H. titia* in allopatry, we have not been able to test for a character displacement pattern in the traditional way by comparing sympatric and allopatric populations. Instead (in a previous study), we tested for wing color displacement in relation to relative species abundance. This approach is based on the assumption that selection to diverge from

Table 3

Comparison of territory canopy cover between sympatric species

Sympatric species	Site	Visit	%Canopy cover species 1	SE	%Canopy cover species 2	SE	z	P
Hetaerina americana/*H. titia*	AR	July 2005	0.441(25)	0.029	0.429(30)	0.043	−0.423	0.673
H. americana/*H. titia*	AR	April 2008	0.152(29)	0.026	0.19(71)	0.016	−1.432	0.152
H. americana/*H. titia*	CV	April 2008	0.764(16)	0.034	0.774(27)	0.02	0.427	0.669
H. americana/*H. titia*	CV	August 2008	0.647(43)	0.022	0.703(32)	0.014	−0.943	0.346
H. occisa/*H. titia*	OT	June 2006	0.532(25)	0.048	0.459(75)	0.029	1.15	0.25
H. occisa/*H. titia*	OT	June 2007	0.477(38)	0.049	0.538(50)	0.043	−0.703	0.482
H. occisa/*H. titia*	PA	July 2007	0.41(52)	0.025	0.393(28)	0.034	0.741	0.458
H. americana/*H. cruentata*	EL	May 2008	0.159(51)	0.032	0.478(24)	0.042	−4.952	<0.001
H. occisa/*H. cruentata*	CT	July 2006	0.072(39)	0.035	0.257(48)	0.015	−5.451	<0.001

Mean canopy cover estimates (proportion) for each species are shown along with sample sizes (number of males). *P* values are based on Wilcoxon rank-sum tests. SE, standard error.

Table 4

Comparison of territory current speed between sympatric species

Sympatric species	Site	Visit	Mean current species 1	SE	Mean current species 1	SE	z	P
H. americana/H. titia	AR	July 2005	3.02(25)	0.083	2.658(30)	0.116	1.933	0.053
H. americana/H. titia	AR	April 2008	2.997(29)	0.003	2.903(71)	0.034	1.294	0.196
H. americana/H. titia	CV	April 2008	2.841(16)	0.151	3.037(27)	0.065	−1.432	0.152
H. americana/H. titia	CV	August 2008	2.974(43)	0.068	2.959(32)	0.067	0.131	0.896
H. occisa/H. titia	OT	June 2006	2.855(25)	0.053	2.668(75)	0.059	1.315	0.189
H. occisa/H. titia	OT	June 2007	2.865(38)	0.05	2.695(50)	0.075	1.483	0.138
H. occisa/H. titia	PA	July 2007	2.283(52)	0.062	2.346(28)	0.096	−0.584	0.559
H. americana/H. cruentata	EL	May 2008	2.813(51)	0.067	3.027(24)	0.031	−0.582	0.561
H. occisa/H. cruentata	CT	July 2006	2.877(39)	0.085	3.326(48)	0.073	3.762	<0.001

Mean current speed estimates for each species are shown along with sample sizes (number of males). Current speed estimates were made on a categorical scale-0: still; 1: moving but flat; 2: shallow standing waves; 3: rippling/mixing; and 4: turbulent/white water. *P* values are based on Wilcoxon rank-sum test.

sympatric congeners is most intense at sites where the focal species is numerically subordinate (Goldberg and Lande 2006). We found, as predicted, that *H. titia* has larger black spots and smaller red wing spots at sites where *H. americana* or *H. occisa* is numerically dominant compared with sites where these congeners are less abundant than *H. titia* (Anderson and Grether 2010b). *Hetaerina americana* shows the predicted inverse pattern, shifting toward larger red wing spots where *H. titia* is numerically dominant. Although patterns of character displacement in secondary sexual characters, such as these, are usually hypothesized to result from selection against cross-species mating (i.e., reproductive character displacement), we are not aware of any evidence that male coloration influences female choice/resistance in *Hetaerina*. By contrast, there is considerable evidence that wing coloration influences interspecific fighting (Anderson and Grether 2010a; this study). Thus, the current study strengthens the evidence for agonistic character displacement in *Hetaerina* damselflies (Grether et al. 2009; Anderson and Grether 2010b).

Promising future work includes removal experiments (Martin PR and Martin TE 2001; Tynkkynen et al. 2006) to confirm the ecological and fitness consequences of species coexistence. We predict that conspecific removals will have dramatic effects on patterns of space use and territory tenure, whereas heterospecific removals will have only a modest effect, if any, in *Hetaerina* species pairs with dissimilar coloration. Removal experiments may also help disentangle whether differing habitat utilization between sympatric species is a cause or a consequence of the frequency of interspecific fighting in species pairs that are similar in coloration. Finally, common garden-type experiments will be required to determine whether shifts in sympatric competitor recognition, habitat preferences, and even wing coloration reflect genetic or plastic responses to the presence of the other species.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco .oxfordjournals.org/.

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