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Character displacement in the fighting colours of *Hetaerina* damselflies

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Aggression between species is a seldom-considered but potentially widespread mechanism of character displacement in secondary sexual characters. Based on previous research showing that similarity in wing coloration directly influences interspecific territorial aggression in *Hetaerina* damselflies, we predicted that wing coloration would show a pattern of character displacement (divergence in sympatry). A geographical survey of four *Hetaerina* damselfly species in Mexico and Texas showed evidence for character displacement in both species pairs that regularly occurs sympatrically. *Hetaerina titia*, a species that typically has large black wing spots and small red wing spots, shifted to having even larger black spots and smaller red wing spots at sites where a congener with large red wing spots is numerically dominant (*Hetaerina americana* or *Hetaerina occisa*). *Hetaerina americana* showed the reverse pattern, shifting towards larger red wing spots where *H. titia* is numerically dominant. This pattern is consistent with the process of agonistic character displacement, but the ontogenetic basis of the shift remains to be demonstrated.

Keywords: agonistic character displacement; interspecific aggression; heterospecific aggression; species recognition; competitor recognition

1. INTRODUCTION

Most studies of character displacement start with the observation of a geographical pattern (e.g. divergence in sympatry). When the displaced trait is a secondary sexual character, the pattern is usually hypothesized to result from selection against cross-species mating, i.e. reproductive character displacement (reviewed in Coyne & Orr 2004; Pfennig & Pfennig 2009). A seldom considered alternative is that such patterns may result from selection against interspecific aggression. Just as traits that play a role in mate choice within species are likely to become targets of selection for improved mate recognition when closely related species that still use similar sexual signals come into secondary contact, traits that play a role in intrasexual competition are likely to become targets of selection for improved competitor recognition when closely related species that still use similar agonistic signals come into secondary contact (Grether *et al.* 2009). Also just as selection against cross-species mating can cause species to diverge in both sexual signals and mate recognition functions (Coyne & Orr 2004; Lemmon *et al.* 2004), selection against interspecific aggression can cause species to diverge in both agonistic signals and competitor recognition functions. For lack of a suitable existing term, Grether *et al.* (2009) introduced the term agonistic character displacement for cases in which character displacement is driven by interspecific aggression. This is a distinctly different evolutionary process than either reproductive or ecological

character displacement (reviewed in Grether *et al.* 2009). Empirically, however, reproductive and agonistic character displacement can be difficult to distinguish, because many secondary sexual characters are involved in both intrasexual competition and mate choice (Berglund *et al.* 1996).

Prior to documenting any geographical patterns, we identified *Hetaerina* damselflies as a system in which agonistic character displacement is likely to be operating in the absence of reproductive character displacement. Male wing coloration in *Hetaerina* is intrasexually selected and does not appear to be a target of female mate choice (Grether 1996; Cordoba-Aguilar *et al.* 2009b). Males of all species in the genus have conspicuous red wing coloration and compete to defend small mating territories in areas with flowing water; females visit these areas to mate and oviposit in submerged vegetation (Garrison 1990). *Hetaerina* territories are similar to the display courts of classic lekking species in that all activities besides mating (feeding, egg-laying, roosting, etc.) usually take place elsewhere (Grether & Grey 1996; Grether & Switzer 2000). Unlike classic lekking species, mating is semi-coercive; males do not perform courtship displays, and although female cooperation is required for copulation, males can clasp and detain resistant females (Grether 1996). Unlike damselflies with resource-based territories (e.g. *Calopteryx* spp.), males do not control access to oviposition sites within their territories and females only rarely oviposit inside their mate's territory (Alcock 1987; Weichsel 1987; Grether 1996; Cordoba-Aguilar *et al.* 2009b). Thus, a *Hetaerina* territory is best viewed as a defended air space within which the resident male has the priority of access to arriving females (Weichsel 1987; Grether 1996). In the defence of a territory, a damselfly may incur many costs, including energetic/physiological costs, injury costs, opportunity

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costs and predation costs (reviewed in Suhonen *et al.* 2008).

Logically, males of different *Hetaerina* species should not waste time and energy fighting over space. Nevertheless, interspecific territorial fights occur frequently between some sympatric species pairs. The proximate reason for this seemingly maladaptive behaviour might be that territory holders simply are unable to discriminate visually between conspecific and heterospecific intruders. Male wing coloration is the most conspicuous marker of species identity (Garrison 1990), and heterospecific fights occur more often between sympatric species that are similar in wing coloration (e.g. *Hetaerina americana* and *Hetaerina occisa*) than between species that are more divergent in wing coloration (e.g. *Hetaerina titia* and *H. occisa*) (Anderson & Grether 2010). In simulated territory intrusion tests with live tethered intruders, residents do not discriminate between conspecific and heterospecific intruders with similar wing coloration but show reduced aggression towards heterospecific intruders with divergent wing coloration (Anderson & Grether 2010). That wing coloration, *per se*, affects how territory residents respond to conspecific and heterospecific intruders has been demonstrated experimentally. Specifically, *H. titia*, a species with red and black wing spots, responds more aggressively to *H. americana* and *H. occisa* intruders with artificial black wings spots than to normal *H. americana* and *H. occisa*, which only have red wing spots. The same wing colour manipulation reduces aggression from *H. americana* and *H. occisa* territory holders, but only at sites where *H. titia* is present (Anderson & Grether 2010). The consistent difference in how males respond to wing colour-manipulated intruders at sympatric and allopatric sites provides clear evidence for agonistic character displacement because shifts in competitor recognition are not predicted under other character displacement processes (Anderson & Grether 2010). Here, we test for corresponding geographical shifts in male wing coloration across a broad swath of the range in which *H. titia* can be found in sympatry with *H. americana* and *H. occisa*.

Multiple geographical patterns have been presented as evidence for character displacement (reviewed in Schluter 2000; Pfennig & Pfennig 2009). The classic approach is to compare trait divergence in areas of sympatry with that in areas of allopatry (Dobzhansky 1937; Grant 1972; Waage 1979; Coyne & Orr 2004; Kirschel *et al.* 2009). An alternative approach is to compare sympatric populations that vary in the relative abundance of heterospecifics (Pfennig & Murphy 2002; Tynkynen *et al.* 2004; Goldberg & Lande 2006; Fisher & Rosenthal 2010). The predicted pattern is that displacement of the trait in one species should be greater where the relative abundance of the other species is greater. One advantage of this approach is that it can be applied to species like *H. titia* that rarely, if ever, occur in allopatry. In general, when testing for geographical patterns indicative of character displacement, it is important to control for environmental factors other than the presence or abundance of heterospecifics (Goldberg & Lande 2006). A geographical pattern suggesting character displacement could merely reflect the outcome of species evolving independently to environmental changes (Endler 1986; Schluter 2000). Alternatively, adaptation along an environmental gradient could cause species to differ

more in allopatry than in sympatry, even if character displacement is occurring (Goldberg & Lande 2006). If body size varies seasonally, as is often the case in damselflies (Grether 1995; Corbet 1999; Cordoba-Aguilar *et al.* 2009a), then character displacement could be obscured. With these considerations, we tested for geographical shifts in male *Hetaerina* wing coloration in relation to the presence or relative abundance of congeners while controlling for clinal and temporal variation.

2. MATERIAL AND METHODS

(a) *Study species and sites*

Hetaerina is endemic to the Americas and reaches its highest diversity in the South American tropics (Garrison 1990). Males of most species in the genus have red spots at the base of all four wings. *Hetaerina titia* is unusual in having red spots only on the forewings and black (melanin) pigmentation on the hindwings and sometimes on the forewings. The black pigmentation is extremely variable in areal coverage, from only a fraction of the wing base to the entire hindwing and most of the forewing. The high level of variation in *H. titia* makes it a good candidate for character displacement, but this species rarely, if ever, occurs in allopatry. We therefore designed our study to make comparisons among sites where *H. titia* occurs with one or more congeners at different relative densities. We also measured these congeners at sites where *H. titia* is absent, to test for character shifts in them using the classic approach. Within sympatric sites, *H. titia* is syntopic (*sensu* Rivas 1964) with *H. americana* and *H. occisa*, that is, males of the different species defend territories in the same stretches of river. Between 2004 and 2008, we collected data at 45 sites where one or more of these three species was present (for locations and sampling dates, see electronic supplementary material, table S1). *Hetaerina titia*, *H. americana* and *H. occisa* were present at 18, 28 and 25 sites, respectively. It was uncommon for all three species to be found together (five sites). A fourth species, *Hetaerina cruentata*, was found at eight of the 45 sites, but never with *H. titia*. The distance between the closest sites ranged from 1.5 to 2105 km, with a median distance (\pm interquartile range) of 20.9 ± 13.5 km.

(b) *Data collection*

Species composition and estimates of relative abundance for each site were based on surveys conducted during May–September, which falls within the peak flight seasons of all *Hetaerina* species in the geographical region sampled (Gonzalez Soriano 1996; Abbott 2005). Each survey covered 200–500 m of suitable riparian habitat and was carried out under weather conditions favourable to damselfly territorial and mating behaviour (full sun or lightly clouded; air temperature in the shade greater than 21 °C). During surveys, we caught damselflies using aerial nets, identified them to species based on clasper morphology (Garrison 1990) and photographed the right fore- and hindwings of mature males using a digital camera (Canon 10D) equipped with a 100 mm macro lens and a macro flash attachment (Canon MT-24EX). Total wing area and the areas covered with red and black pigment were measured manually on a computer screen using NIH Image. Wing spot areas were divided by wing area prior to analysis. Each wing measurement was assigned a Julian date corresponding to the first day of the corresponding survey.

Table 1. Evidence for character displacement in the wing coloration of *H. titia*. Analysis of geographical variation in (a) *H. titia* red pigmentation, (b) *H. titia* black pigmentation, and (c) *H. titia* wing area.

model term	Sympatric congener								
	<i>H. americana</i>			<i>H. occisa</i>			either species		
	<i>B</i>	<i>z</i>	<i>p</i>	<i>B</i>	<i>z</i>	<i>p</i>	<i>B</i>	<i>z</i>	<i>p</i>
(a) Sources of variation in <i>H. titia</i> red wing spot area									
relative abundance	-0.0461	-2.80	0.005	-0.0525	-1.93	0.053	-0.0257	-2.07	0.039
sampling date	0.0006	17.84	<0.001	-0.0002	-1.76	0.078	0.0006	20.66	<0.001
latitude	-0.0030	-1.53	0.127	0.0146	1.03	0.303	-0.0056	-3.79	<0.001
longitude	-0.0089	-3.59	<0.001	—	—	—	-0.0067	-3.50	<0.001
<i>R</i> ²		0.696			0.268			0.7292	
individuals, sites		632, 13			619, 10			1000, 18	
(b) <i>H. titia</i> black wing spot area									
relative abundance	0.1273	3.08	0.002	0.2929	2.70	0.007	0.0904	4.28	<0.001
sampling date	0.0028	28.20	<0.001	0.0039	3.17	0.002	0.0028	17.84	<0.001
latitude	-0.0102	-2.09	0.036	-0.1506	-2.53	0.011	-0.0107	-6.60	<0.001
longitude	0.0468	7.59	<0.001	—	—	—	0.0495	41.79	<0.001
<i>R</i> ²		0.7609			0.1622			0.6691	
individuals, sites		630, 13			618, 10			998, 18	
(c) <i>H. titia</i> wing area									
relative abundance	15.6582	1.82	0.069	14.5040	0.82	0.409	7.4818	0.81	0.418
sampling date	0.2313	9.25	<0.001	0.0246	0.15	0.878	0.2245	8.55	<0.001
latitude	-1.0851	-1.10	0.273	-4.0119	-0.43	0.667	-0.0475	-0.04	0.965
longitude	5.3042	4.24	<0.001	—	—	—	4.4142	3.16	0.002
<i>R</i> ²		0.367			0.1224			0.5121	
individuals, sites		634, 13			622, 10			1004, 18	

A subset of sites were selected for detailed behavioural investigation reported elsewhere (Anderson & Grether 2010) and the relative abundance of each *Hetaerina* species from these surveys may be estimated robustly. Other sites included in the current dataset were visited on a single day and thus our estimates of species abundance from these surveys are necessarily less precise. To assess the reliability of these single-day surveys, we examined our records from the first day of surveys carried out at the more intensively studied sites. The rank order of relative species abundance was reliably determined on the first day, but it took several days to obtain reliable quantitative estimates of relative abundance. At sites that we visited repeatedly, the rank order of relative abundance did not change during the peak flight season (May–September). Therefore, for the analyses presented here, we use a binary measure of relative abundance, classifying one species at a site as ‘most common’ and the others as ‘less common’ during the peak flight season.

The effects of the relative abundance of heterospecifics on *H. titia* wing morphology were investigated in separate models with *H. americana* or *H. occisa* designated as the species of heterospecific. Furthermore, we used all of the *H. titia* sites by testing a model that allowed either *H. americana* or *H. occisa* to serve as a heterospecific and we pooled the relative abundances if both were present. At every site composed of all three species, we were able to unambiguously define a single species as most common, regardless of whether the relative abundance of heterospecifics was pooled or not.

(c) Data analysis

We constructed random-effects generalized least-squares regression models separately for each species and dependent

variable, with site entered as a clustered variable (xtreg in STATA 10.0; StataCorp, College Station, TX, USA). The dependent variables were wing area, the relative area of red pigmentation or the relative area of black wing pigmentation (*H. titia* only). All models initially included the covariates Julian date, latitude and longitude. In cases where longitude and latitude were collinear, the longitude term was dropped. The effects of relative species abundance and sympatry versus allopatry were tested by including binary terms in the models. All final models had variance inflation factors less than five and thus did not suffer from multi-collinearity (Afifi *et al.* 2004). Normal probability plots showed the residuals to be approximately normal.

3. RESULTS

As predicted, *H. titia* had more black wing pigmentation, and less red wing pigmentation, at sites where *H. americana* or *H. occisa* was the most abundant species, compared with sites where *H. titia* was the most abundant species (table 1). These trends held and remained statistically significant when the analysis was restricted to the subset of sites where *H. titia* occurs with *H. americana* and relative abundance was based on just this species pair (figure 1 and table 1). The same was true when the analysis was restricted to the subset of sites where *H. titia* occurs with *H. occisa*, except that the effect of relative abundance on the red wing spots of *H. titia* was only marginally significant (table 1 and figure 1). None of these patterns can be attributed to body size variation because *H. titia* wing area was not correlated with the relative abundance of heterospecifics (table 1).

Allopatry versus sympatry comparisons revealed no evidence for character displacement in the wing

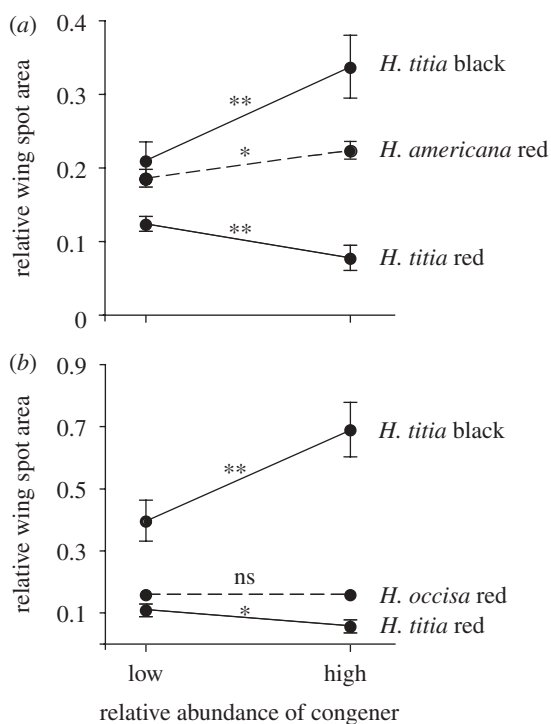


Figure 1. Character displacement patterns in *Hetaerina* wing coloration. (a) Character shifts in zones of sympatry between *H. americana* and *H. titia*; (b) character shifts in zones of sympatry between *H. occisa* and *H. titia*. LS means \pm s.e. from the regression models of tables 1–3 are shown. * $p < 0.05$, ** $p < 0.01$.

coloration of *H. americana* or *H. occisa* (table 2). The only evidence for a sympatry effect is that *H. americana* had significantly larger wings where *H. titia* was present, compared with sites where *H. titia* was absent (electronic supplementary material, figure S1). Nevertheless, when the analysis was restricted to sympatric sites, a character displacement pattern emerged in the wing coloration of *H. americana* (figure 1). The red wing spots of *H. americana* were significantly larger at sites where *H. titia* was the most abundant species ($p = 0.024$; table 3). *Hetaerina titia* abundance was not a significant predictor of the size of the red wing spots on *H. occisa* or the wing areas of either species.

Independent of the presence/absence or relative abundance of heterospecifics, most wing characters showed significant clinal and/or temporal variation (tables 1–3; electronic supplementary material, table S2). The proportion of the wing covered with pigment increased with Julian date in all four species, including *H. cruentata*. Wing area also increased with Julian date in *H. americana* and *H. titia*, but not in the other two species.

4. DISCUSSION

We tested for character displacement patterns in the wing coloration of the damselfly *H. titia* in relation to two of its sympatric congeners, *H. americana* and *H. occisa*, and vice versa. *Hetaerina titia* is a good candidate for character displacement because its black and red wing coloration is highly variable and some variants resemble the sympatric congeners, which only have red wing coloration, more closely than others. Our dataset for testing for character

shifts in *H. titia* consisted of morphological measurements and estimates of relative species abundance from 18 sites where *H. titia* occurs with one or both congeners. We predicted that *H. titia* would shift towards having more black wing coloration and less red wing coloration at sites dominated by the congeners, in comparison to sites where *H. titia* is numerically dominant. Both predictions were upheld (figure 1). When the same approach was used to test for character shifts in the congeners in relation to the abundance of *H. titia*, *H. americana* showed the predicted character shift, i.e. larger red wing spots where *H. titia* is numerically dominant, but *H. occisa* did not (figure 1). Classic sympatry versus allopatry comparisons did not detect effects of the presence of *H. titia* on the wing coloration of either congener. Thus, we obtained consistent evidence for character displacement patterns in *H. titia*, no such evidence in *H. occisa* and mixed evidence in *H. americana*.

While it is not possible to robustly infer evolutionary mechanisms from geographical patterns, we have independent evidence for agonistic character displacement in this system. In simulated territory intrusion tests, putting black ink on the hindwings of *H. americana* or *H. occisa* intruders reduced the rate of mid-air attacks by territory holders of the same species. Furthermore, intruders with fully black hindwings were attacked less often than intruders with half-black hindwings. These graded responses to black wing coloration were found only at sites where *H. titia* is present, which suggests that they evolved in response to aggressive interference with *H. titia* (Anderson & Grether 2010). Thus, the observed shifts in the black wing coloration of *H. titia* are a predicted consequence of selection imposed by its congeners in an aggressive context. The effects of red wing spot size on heterospecific aggression have not been investigated experimentally, but the observed geographical shifts in the red wing spots of *H. titia* and *H. americana* are in the directions predicted by the agonistic character displacement hypothesis. It is possible that reproductive interference (i.e. reproductive character displacement) has also contributed to these geographical patterns, but we are not aware of any evidence that male coloration influences female choice/resistance in *Hetaerina*.

It is not clear why we did not also observe a character displacement pattern in *H. occisa*, but there are several possible reasons. Selection on *H. occisa* might be weak because this species is behaviourally dominant to *H. titia*, *H. occisa* wing spot size might be insufficiently heritable to respond to selection or gene flow might have obscured past responses to selection. We have no basis, as yet, for distinguishing among these alternatives. In general, there are many possible reasons why character displacement patterns might not be observed (Goldberg & Lande 2006).

Common garden experiments will be required to firmly establish whether the observed geographical shifts in wing coloration reflect genetic differentiation. *Hetaerina* wing coloration does not become developmentally fixed until about two weeks post-emergence (Grether 1996), so it is conceivable that the competitive environment experienced by males as larvae or as maturing adults influences wing spot development. Upon repeated visits to sites in the peak flight season, we

Table 2. Analysis of geographical variation in *H. americana* and *H. occisa* red pigmentation and wing area from sites sympatric with *H. titia* and sites allopatric with *H. titia*.

focal species		<i>H. americana</i>						<i>H. occisa</i>					
model term	<i>B</i>	<i>z</i>	<i>p</i>	wing area			red spot area			wing area			
				<i>B</i>	<i>z</i>	<i>p</i>	<i>B</i>	<i>z</i>	<i>p</i>	<i>B</i>	<i>z</i>	<i>p</i>	
sympatry w/ <i>H. titia</i>	0.0125	1.08	0.282	13.1135	2.26	0.024	0.0021	1.16	0.246	1.3630	0.21	0.83	
sampling date	0.0012	41.82	<0.001	0.1423	5.49	<0.001	0.0004	10.30	<0.001	0.0833	1.30	0.194	
latitude	0.0023	1.81	0.07	3.3952	5.56	<0.001	-0.0085	-3.71	<0.001	0.6568	0.13	0.897	
longitude	0.0046	3.60	<0.001	3.2608	5.41	<0.001	-0.0021	-4.50	<0.001	-1.2361	-0.96	0.339	
R^2		0.7258			0.6139			0.3287			0.0541		
individuals, sites		874, 28			877, 28			414, 25			414, 25		

Table 3. Analysis of geographical variation in *H. americana* and *H. occisa* red pigmentation and wing area from sites sympatric with *H. titia*.

focal species		<i>H. americana</i>						<i>H. occisa</i>					
model term	<i>B</i>	<i>z</i>	<i>p</i>	wing area			red spot area			wing area			
				<i>B</i>	<i>Z</i>	<i>p</i>	<i>B</i>	<i>z</i>	<i>p</i>	<i>B</i>	<i>z</i>	<i>p</i>	
relative abundance	0.0378	2.26	0.024	-6.7861	-1.26	0.208	-0.0003	-0.06	0.954	7.3138	1.05	0.294	
sampling date	0.0012	38.74	<0.001	0.1316	5.28	<0.001	0.0005	5.68	<0.001	0.1320	1.51	0.132	
latitude	0.0043	2.38	0.017	5.1090	9.16	<0.001	-0.0108	-3.04	0.002	1.7674	0.42	0.674	
longitude	0.0029	1.01	0.314	-0.8022	-0.81	0.417	-0.0022	-2.65	0.008	-1.1850	-1.06	0.289	
R^2		0.6818			0.5671			0.3612			0.2113		
individuals, sites		523, 13			524, 13			213, 10			213, 10		

consistently classified the same species as most common. We do not know, however, whether present-day measures of species' abundance reflect the relative abundance over evolutionary time. If population densities and relative abundance have varied over evolutionary time, this seems likely to promote a role for plasticity in generating this character displacement pattern (reviewed in Pfennig & Pfennig 2009). If the geographical shifts in wing coloration that we have documented here reflect a plastic response to the presence of other *Hetaerina* species, as opposed to genetic divergence in mean trait values, this could be an example of facultative character displacement (Pfennig & Murphy 2002). Demonstrating facultative character displacement would require showing that plasticity itself is displaced among populations. A final possibility is that a reaction norm common to all populations mediates the shift to the presence of the other species.

Ours is the second study to document a character displacement pattern in damselfly fighting colours. Tynkkynen *et al.* (2004) showed that the average size of the wing spots on male *Calopteryx splendens* decreased as the relative abundance of *Calopteryx virgo* increased across 23 sites in Finland. Interspecific territorial aggression (i.e. agonistic character displacement) was implicated as the mechanism responsible for this character displacement pattern because competitively dominant *C. virgo* males are most aggressive to *C. splendens* males with large wing spots (Tynkkynen *et al.* 2004, 2006). Selection against hybridization (i.e. reproductive character displacement) is a plausible alternative mechanism, however, because these species are known to hybridize and female *C. splendens* have been reported to choose mates on the basis of wing coloration (Siva-Jothy 1999; Cordoba-Aguilar 2002; Svensson *et al.* 2006, 2007).

We also found evidence for temporal and clinal variation in *Hetaerina* wing pigmentation, independent of the abundance of heterospecifics. The relationships between wing measurements and latitude/longitude were species specific, but relative wing spot sizes increased with Julian date in all four *Hetaerina* species investigated (tables 1–3; electronic supplementary material, table S2). This corroborates the work of Cordoba-Aguilar *et al.* (2009a), who also reported seasonal variation in spot expression of *H. americana*, and extends the finding to other *Hetaerina* species. In our study, the relationship between wing spot size and Julian date was approximately linear, but perhaps only because our samples were collected between late spring and late summer. Cordoba-Aguilar *et al.* (2009a) sampled throughout the year and found a peak in wing spot size in late summer, with smaller wing spots in spring and fall samples. Julian date probably correlates with, and therefore summarizes the influence of, multiple proximate environmental influences on wing spot size, such as temperature and the duration of larval development.

Our results suggest that, in the absence of interspecific aggression, the wing coloration of *H. titia* would be more similar to that of its congeners. An intriguing, albeit speculative, hypothesis is that melanin wing pigmentation was an evolutionary innovation that enabled *H. titia* to expand its range into regions where other *Hetaerina* species were already well established. This species has one of the broadest ranges of any *Hetaerina* spp.

and coexists in microsympatry with several different congeners. Moreover, a territory mapping study revealed that *H. titia* territories were overdispersed in relation to the territories of sympatric congeners, while the reverse was observed in other species pairs (C. N. Anderson & G. F. Grether 2010, unpublished data). Although melanin wing pigmentation has been suggested to serve intraspecific, intrasexual signalling functions in *H. titia* (Cordoba-Aguilar *et al.* 2007), the intraspecific functions could have evolved secondarily. In any case, our inference that the observed wing colour shifts in *H. titia* are products of interspecific aggression does not depend on the evolutionary origins of melanin wing pigmentation.

Interest in the role of interspecific aggression in character displacement is rapidly growing (e.g. Adams 2004; Seehausen & Schluter 2004; Albert *et al.* 2007; reviewed in Grether *et al.* 2009). Many of these studies, like the one reported here, report patterns that are consistent with the process of agonistic character displacement. We have documented evidence for the process of agonistic character displacement in the *Hetaerina* system, by experimentally demonstrating the direct role that wing coloration plays in territorial responses to conspecific and heterospecific intruders (Anderson & Grether 2010). Future work ought to investigate the fitness consequences (e.g. reduced survival or lifetime reproductive success) of interspecific aggression (e.g. Tynkkynen *et al.* 2005). Furthermore, ruling out alternative processes that may generate similar patterns is a crucial direction for future research.

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