Interspecific aggression and character displacement of competitor recognition in *Hetaerina* damselflies

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In zones of sympatry between closely related species, species recognition errors in a competitive context can cause character displacement in agonistic signals and competitor recognition functions, just as species recognition errors in a mating context can cause character displacement in mating signals and mate recognition. These two processes are difficult to distinguish because the same traits can serve as both agonistic and mating signals. One solution is to test for sympatric shifts in recognition functions. We studied competitor recognition in *Hetaerina* damselflies by challenging territory holders with live tethered conspecific and heterospecific intruders. Heterospecific intruders elicited less aggression than conspecific intruders in species pairs with dissimilar wing coloration (*H. occisa/H. titia, H. americana/H. titia*) but not in species pairs with similar wing coloration (*H. occisa/H. cruentata, H. americana/H. cruentata*). Natural variation in the area of black wing pigmentation on *H. titia* intruders correlated negatively with heterospecific aggression. To directly examine the role of wing coloration, we blackened the wings of *H. occisa* or *H. americana* intruders and measured responses of conspecific territory holders. This treatment reduced territorial aggression at multiple sites where *H. titia* is present, but not at allopatric sites. These results provide strong evidence for agonistic character displacement.

Keywords: character displacement; agonistic character displacement; species recognition; competitor recognition; mate recognition; intrasexual competition

1. INTRODUCTION

When closely related species come into secondary contact, species recognition errors may arise in both intrasexual and intersexual contexts. Similarity between species in traits used in mate recognition can lead to maladaptive cross-species courtship and mating (Estrada & Jiggins 2008; Groening & Hochkirch 2008). Selection resulting from such mate recognition errors can cause mating signals and/or mate preferences to diverge in areas of sympatry compared with areas of allopatry, a process known as reproductive character displacement (RCD; Brown & Wilson 1956). Because multiple processes can lead to similar geographical patterns in mating signals, it is important to also test for shifts in mate preferences when evaluating putative cases of RCD (Gabor & Ryan 2001). Several studies have documented sympatric shifts in both mating signals and mate preferences (e.g. Ryan *et al.* 1996; Pfennig 2000; Ptacek 2000; Hobel & Gerhardt 2003; Hoskin *et al.* 2005; Rundle *et al.* 2005; Higgie & Blows 2008; Lemmon 2009).

A less explored consequence of secondary contact is the possibility of maladaptive interspecific aggression arising from similarity between species in traits used for recognizing conspecific competitors. Selection resulting from errors in competitor recognition can cause divergence in agonistic signals and/or competitor recognition

functions between species in sympatry, a process known as agonistic character displacement (ACD; Grether *et al.* 2009). There are many putative examples of divergent ACD, but most involve displacement in traits that might also be subject to RCD (reviewed in Grether *et al.* 2009). For example, male pied flycatchers (*Ficedula hypoleuca*) occur in both brown and black morphs; the black morph resembles the competitively dominant collared flycatcher (*F. albicollis*). Males of the brown morph receive less territorial aggression from collared flycatchers than the black morph and are only found where the two species occur in sympatry (Alatalo *et al.* 1994). This geographical pattern has the effect of reducing competitive interference between the species in sympatry, but selection against cross-species mating provides a plausible alternative explanation. Indeed, while female pied flycatchers prefer black males over brown males at allopatric sites, this preference is reversed at sympatric sites (Saetre *et al.* 1997; Saether *et al.* 2007).

In another illustrative example, the average wing spot size of the damselfly *Calopteyrx splendens* decreases across populations with increasing relative abundance of the large-wing-spotted and competitively dominant congener, *C. virgo*, perhaps because territorial aggression from *C. virgo* increases with the size of the black spots on male *C. splendens* (Tynkkynen *et al.* 2004, 2005, 2006). Selection against cross-species mating offers a plausible alternative explanation for the geographical pattern, however, because female *C. splendens* appear to use wing pigmentation to discriminate between conspecific and heterospecific males (Svensson *et al.* 2007).

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Many secondary sexual characters are used in both mate choice and mate competition contexts (reviewed in Berglund *et al.* 1996). Therefore, sympatric shifts in such signals (coloration, song, etc.) are often difficult to attribute to RCD or ACD alone. Sympatric shifts in recognition functions do not suffer from this ambiguity because they involve different behavioural contexts and have distinctly different outcomes (i.e. reductions in cross-species mating versus reductions in interspecific aggression). We tested for sympatric shifts in competitor recognition in the damselfly genus *Hetaerina*.

Territorial aggression between sympatric *Hetaerina* is best understood as a case of misidentification (Murray 1981; Schultz & Switzer 2001) rather than adaptive defence of a shared limiting resource (Cody 1969), as the following details of this system will clarify. Adult males compete for small mating territories along river margins where females oviposit (Alcock 1987; Weichsel 1987; Grether 1996). These clearly are not feeding territories because foraging activity peaks in the hours before males arrive on their territories in the morning and again after they abandon defence in the late afternoon (Grether & Grey 1996). Mating is initiated by tandem formation, in which the male clasps the female's prothorax using his caudal abdominal appendage (Corbet 1999; Cordoba-Aguilar & Cordero-Rivera 2005). Tandem pairs typically fly through multiple territories and only rarely oviposit in the territory of the female's mate (Grether 1996). Residents do not prevent tandem pairs from using oviposition sites (submerged vegetation) within their territories and do not abandon their territories if oviposition substrate is experimentally removed (Alcock 1987). Thus, a *Hetaerina* territory is essentially defended air space within which the resident male has priority of access to passing females (Weichsel 1987; Grether 1996). Logically, in such a system, males of different species should not waste time and energy competing for space.

We studied four sympatric species pairs—*Hetaerina americana/H. titia*, *H. americana/H. cruentata*, *H. occisa/H. titia* and *H. occisa/H. cruentata*—as well as allopatric populations of *H. americana* and *H. occisa*. Males of all species have red spots at the base of each wing, and the wing spot size is intrasexually selected, at least in *H. americana* (Grether 1996; Contreras-Garduno *et al.* 2006; Serrano-Meneses *et al.* 2007). Beyond the basal red spots, the wings of *H. americana*, *H. occisa* and *H. cruentata* are mostly transparent; *H. occisa* wings bear small apical red spots and the wing tips of *H. cruentata* are darkened (Westfall & May 1996). In contrast, *H. titia* has extensive black pigmentation on the wings (Cordoba-Aguilar *et al.* 2007). *H. titia* is also unique among *Hetaerina* in showing extensive variation in wing pigmentation both within and between populations (Johnson 1963; Garrison 1990). The hindwings of *H. titia* range from black only at the wing base (and otherwise transparent) to completely black, and there is geographical and seasonal variation in the extent of this variation (C. N. Anderson & G. F. Grether 2007, unpublished data). Despite the variation, we usually had no difficulty identifying males to species in the field based on wing coloration alone (verified by examination of abdominal claspers and other taxonomically diagnostic characters; Garrison 1990), but our results indicate that the damselflies themselves have trouble distinguishing species.

Our initial observations revealed that interspecific fights often occur where *Hetaerina* species are found in sympatry. Based on the assumption that interspecific aggression is maladaptive in this system (see above), we predicted that if males are able to distinguish between conspecific and heterospecific intruders, their ability to do so would be affected by variation in wing coloration and enhanced in sympatric compared with allopatric populations of the same species. Here we report the results of simulated intruder tests of two types: (i) tests in which territory holders were presented with tethered but otherwise unmanipulated males of two species; and (ii) tests in which territory holders were presented with conspecific males whose wing coloration was manipulated to resemble a congener and two types of controls (unmanipulated and sham-manipulated conspecifics). The second type of test was carried out at sites where the species occur in sympatry and also at allopatric sites, to test for sympatric shifts in competitor recognition.

2. MATERIAL AND METHODS

(a) *Sampling design and localities*

Our sampling design is based on comparing sites where *H. titia* is sympatric with *H. americana* or *H. occisa* to sites where one of these species is present and *H. titia* is absent. Testing for character displacement patterns by comparing allopatric and sympatric populations requires some level of replication and phylogenetic independence within each of these categories (Schluter 2000). Based on the limited dispersal capabilities of damselflies, we guessed that geographically close populations would be more similar genetically than geographically distant populations and used this as a guide for selecting study sites. For each study site where *H. titia* occurs in sympatry with *H. americana* or *H. occisa*, we also sampled a nearby site where the congener occurs in allopatry from *H. titia*. *Post hoc* genetic analyses confirmed that we succeeded in sampling multiple, independent areas of sympatry (see electronic supplementary material).

The experiments reported here were carried out in June–August 2006, May–July 2007 and April–May 2008 at 10 sites in Texas and Mexico (figure 1): Lampasas, Texas (LP: 31.07 N, 98.17 W); Ingram, Texas (RV: 30.07 N, 99.28 W); 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); southwest of La Tinaja, Mexico (UO: 18.71 N, 96.57 W); southeast of La Tinaja, Mexico (OT: 18.69 N, 96.39 W); Playa Hermosa, Mexico (PH: 18.66 N, 95.13 W); La Palma, Mexico (PA: 18.56 N, 95.07 W); and Tebanca, Mexico (CT: 18.37 N, 95.00 W). From the standpoint of behavioural research, all of these sites are effectively one- or two-species sites, although other *Hetaerina* species were present at low densities at some sites. We visited each site multiple times between 2004 and 2008 and never observed *H. titia* at LP, EL, UO, PH or CT. We therefore classify these sites as allopatric from *H. titia*. *H. cruentata* was present at two of the latter sites (CT and EL). At three sites we performed replicate experiments in separate visits: EL (May 2007, May 2008), CV (April 2008, August 2008) and OT (June 2006, June 2007). In no cases did the results differ within sites between
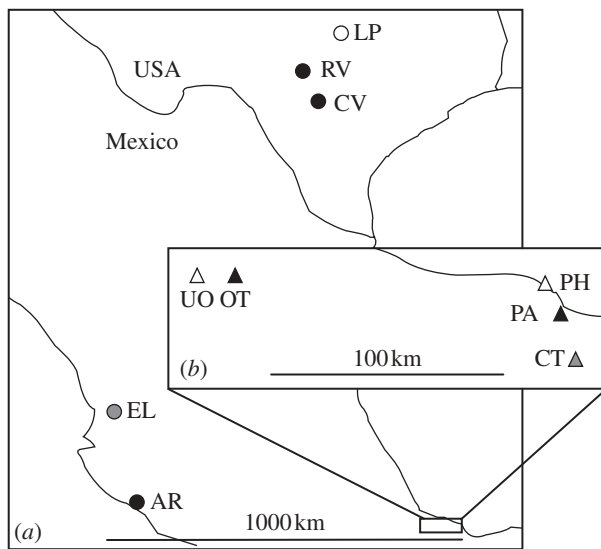
Figure 1. (*a*) Distribution of the five *H. americana* (circles) study sites in Texas and Mexico. (*b*) Distribution of the five *H. occisa* (triangles) study sites in the Mexican state of Veracruz. The study sites were: Lampasas, Texas (LP); Ingram, Texas (RV); Castroville, Texas (CV); El Limon, Mexico (EL); Armeria, Mexico (AR); southwest of La Tinaja, Mexico (UO); southeast of La Tinaja, Mexico (OT); Playa Hermosa, Mexico (PH); La Palma, Mexico (PA); and Tebanca, Mexico (CT). Black triangles, *H. occisa/H. titia*; black circles, *H. americana/H. titia*; grey triangles, *H. occisa/ H. cruentata*; grey circles, *H. americana/H. titia*; white triangles, *H. occisa*; white circles, *H. americana*.

visits at $p < 0.05$; therefore, for most analyses, we present only the pooled results. The one exception (involving site CV) is explained below.

(b) *Experimental set-up and procedure*
Study transects (50–100 m) were established along river margins. Each male damselfly was individually marked with a unique combination of three colours on its abdomen using paint pens. A male was classified as a territory holder if it was consistently seen at the same location (± 1.5 m) on at least two consecutive days during the hours when males are territorial (10.00–18.00 h).

During a simulated territory intrusion test, an observer recorded the behaviour of the territory holder on a continuously running audio recorder, while a tethered male was presented using 0.3 m of fine transparent thread and a modified fishing pole. The pole was manoeuvred to keep the tethered male flying within the focal male's territory for 2 min. Each territory holder was tested with multiple tethered intruders within a 20–30 min window. A minimum of 5 min elapsed between consecutive tests on a territorial male and the order of treatments (see below) was varied systematically. This design allowed each individual territory holder to serve as his own control. Tethered males were captured outside the study transect and used for at most two tests before being released again outside the transect. All simulated intruder tests were carried out between 10.00–18.00 h under sunny or lightly clouded conditions (territory defence wanes under heavy cloudy cover).

The responses of territory holders to tethered intruders covered the full range of behaviours observed in natural territorial encounters. Aggressive responses that we recorded include chasing (flight towards the tethered male) and two forms of escalation that occur during chasing: 'slams', defined as attempts to ram the tethered intruder (whether successful or not), and 'grabs', defined as extended physical contact in which the resident lands on the intruder in flight.

(c) *Responses to unmanipulated conspecific and heterospecific intruders*
The main goal of this experiment, which was carried out on all four sympatric species pairs, was to determine whether territory holders discriminate between conspecific and heterospecific male intruders. Each territory holder was presented sequentially with both types of intruders, with the order of presentation alternating between trials. We conducted paired intrusion tests on 66 *H. americana* territory holders (AR: 16, CV: 33, EL: 17), 58 *H. occisa* territory holders (OT: 16, PA: 26, CT: 16), 79 *H. titia* territory holders (AR: 14, CV: 30, OT: 16, PA: 19) and 25 *H. cruentata* territory holders (CT: 15, EL: 10). A subset of these data was used to examine the effects of natural variation in the wing coloration of *H. titia* intruders on the responses of territory holders. We made an effort to use *H. titia* intruders representing the full range of wing spot variation present at each site.

Hetaernia titia wings were photographed with a digital camera (Canon 10D; Canon USA, Inc., Lake Success, NY, USA) equipped with a 100 mm macro lens and macro flash attachment (Canon MT-24EX). Measurements of total 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 dark pigment.

(d) *Responses to wing-colour-manipulated intruders*
To directly examine the effects of an intruder's wing coloration on the responses of territory holders, while controlling for other traits that differ between species, we manipulated the wing colour of tethered intruders using ink. Specifically, we examined the effects of making the hindwings of *H. americana* and *H. occisa* intruders more closely resemble those of *H. titia*. We measured the responses of *H. americana* and *H. occisa* territory holders to colour-manipulated conspecifics at sites where *H. titia* was both present and absent. If present at a site, we also measured the responses of *H. titia* and *H. cruentata* territory holders.

The wing colour treatments were as follows. Clear: hindwings fully painted with colourless marker (Prismacolor PM-121; Sanford L.P., Oak Brook, IL, USA). Half Black: basal half of hindwings painted with black marker (Prismacolor PM-2) and distal half of hindwings painted with colourless marker. Black: hindwings fully painted with the black marker. The black marker used in this experiment was chosen because it closely matches the colour of *H. titia* wings, both to human eyes and as assessed with reflectance spectrometry. Each territory holder was presented sequentially with intruders of all three treatments, with the order of presentation alternating between trials. We conducted wing-colour-manipulated intrusion tests on 126 *H. americana* territory holders (AR: 16, CV: 33, RV: 17, EL: 44, LP: 16), 102 *H. occisa* territory holders (OT: 32, PA: 26, CT: 15, PH: 14, UO: 15), 103 *H. titia* territory holders (AR: 14, CV: 30, RV: 9, OT: 31, PA: 19) and 35 *H. cruentata* territory holders (CT: 19, EL: 16).

(e) *Data analysis*

Territorial aggression was quantified as the proportion of time the resident spent chasing the tethered intruder and by the rate of mid-air attacks: (number of slams + number of grabs)/trial duration. Attack rate was log $(x + 0.01)$ transformed and proportion of time chasing was arcsine $(\sqrt{x})$ transformed to meet parametric assumptions.

We used paired t-tests to compare the response of territory holders to conspecific and heterospecific intruders, separately by species and site. The effects of natural variation in *H. titia* wing spot size were examined with generalized linear models. Each generalized linear model was fitted with a binomial error structure and logit link function, with the proportion of time chasing the *H. titia* intruder as the response variable, the size of the hindwing spot of the *H. titia* intruder fitted as the explanatory term and the proportion of time chasing the conspecific intruder as a covariate to control for individual variation in aggression. Because mean *H. titia* spot size at site CV differed between visits ($t_{34} = 7.37$, $p < 0.0001$), we present the results for each visit separately.

We used repeated-measures analyses of variance to analyse the results of the colour-manipulated intruder tests for each species separately. For *H. americana* and *H. occisa*, explanatory terms in the full model included the between-subjects factors sympatry with *H. titia* (levels: allopatric, sympatric) and site (nested within sympatry category), and the within-subjects factor intruder wing colour treatment (levels: clear, half, black). The interaction between sympatry status and intruder treatment was of particular interest because this tests for sympatric shifts in the response function. For *H. titia* and *H. cruentata*, all sites were of the same sympatry status, so this term was dropped from the models.

3. RESULTS

(a) *Responses to unmanipulated conspecific and heterospecific intruders*

In most cases, territorial males attacked conspecific intruders at higher rates than heterospecific intruders, but the exceptions are informative (figure 2; table 1). *Hetaerina titia* territory holders were consistently more aggressive to conspecific intruders than to intruders of the sympatric congener, although the results for one site were not significant after a Bonferroni correction for multiple tests (table 1). Territorial *H. americana* and *H. occisa* attacked conspecific intruders more aggressively than *H. titia* intruders but did not distinguish between conspecific intruders and *H. cruentata* intruders. *Hetaerina cruentata* territory holders did not discriminate between conspecific intruders and either *H. americana* or *H. occisa* intruders. Compared with *H. titia*, *H. cruentata* is more similar to *H. americana* and *H. occisa* in wing coloration (figure 2). Therefore, these results support the idea that males use wing coloration to identify conspecific competitors and have difficulty distinguishing between males of similar-looking species.

The proportional size of the hindspot on *H. titia* intruders was negatively related to the duration of chasing by *H. occisa* territorial holders (OT: $z = -4.52$, $p < 0.001$, $n = 15$; PA: $z = -6.31$, $p < 0.001$, $n = 24$) but not by *H. americana* territory holders (AR: $z = 0.58$, $p = 0.559$, $n = 16$; April 2008 CV: $z = -0.61$, $p = 0.539$, $n = 16$; August 2008 CV: $z = -1.60$, $p = 0.111$, $n = 15$).
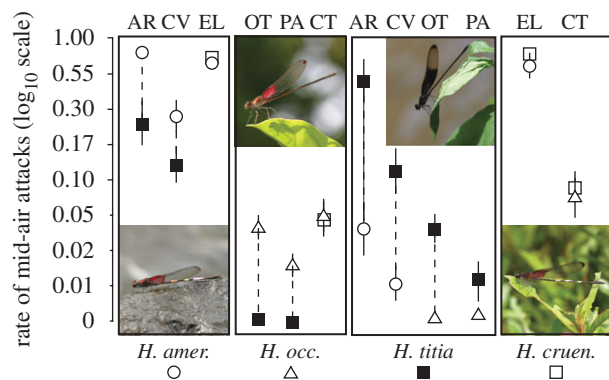

Figure 2. The average attack rate (attacks per second) $\pm$ s.e. of territory holders of four *Hetaerina* spp. (named below graphs and shown in photos) to tethered conspecifics and sympatric congeners. Symbols identify species of the intruders (open circles, *H. americana*; open triangles, *H. occisa*; filled squares, *H. titia*; open squares, *H. cruentata*). Site codes are given above the corresponding symbols. Vertical dashed lines connect means for tests carried out at the same site.

(b) *Responses to wing-colour-manipulated intruders*

For both *H. americana* and *H. occisa*, there was a significant sympatry status by intruder treatment interaction (*H. americana*: $F_{2,248} = 20.91$, $p < 0.001$; *H. occisa*: $F_{2,200} = 22.02$, $p < 0.001$). Analysing sites separately showed that blackening the wings of intruders reduced the aggressive response of conspecific territory holders at sympatric sites (figure 3; *H. americana*: AR: $F_{2,30} = 4.82$, $p = 0.0153$; CV: $F_{2,64} = 30.85$, $p < 0.0001$; RV: $F_{2,32} = 25.03$, $p < 0.001$; *H. occisa*: OT: $F_{2,62} = 78.08$, $p < 0.001$; PA: $F_{2,50} = 6.93$, $p = 0.0022$) but not at allopatric sites (*H. americana*: EL: $F_{2,86} = 2.22$, $p = 0.1151$; LP: $F_{2,30} = 0.90$, $p = 0.4176$; *H. occisa*: CT: $F_{2,28} = 1.27$, $p = 0.2959$; PH: $F_{2,26} = 2.83$, $p = 0.0776$; UO: $F_{2,28} = 1.97$, $p = 0.1584$). The wing colour treatment also had no significant effect on the responses of *H. cruentata* territory holders at sites allopatric from *H. titia* (CT: $F_{2,36} = 1.32$, $p = 0.28$; EL: $F_{2,30} = 0.38$, $p = 0.69$).

In contrast, blackening the wings of heterospecific (*H. occisa* or *H. americana*) intruders increased the attack rate of *H. titia* territory holders at three of five sites (figure 4; AR: $F_{2,26} = 15.64$, $p < 0.001$; CV: $F_{2,58} = 12.00$, $p < 0.001$; OT: $F_{2,60} = 43.08$; $p < 0.0001$; PA: $F_{2,36} = 2.08$, $p = 0.1391$; RV: $F_{2,16} = 2.29$; $p = 0.1339$). The non-significant results for sites PA and RV may reflect the fact that attack rates were low for all types of intruders at these sites. When the same model was tested with proportion of time chasing as the dependent variable, the wing colour treatment had highly significant effects on the response of *H. titia* territory holders at all sites (AR: $F_{2,26} = 8.58$, $p = 0.001$; CV: $F_{2,58} = 8.29$, $p < 0.001$; OT: $F_{2,60} = 49.50$; $p < 0.0001$; PA: $F_{2,36} = 15.28$, $p < 0.001$; RV: $F_{2,16} = 21.12$; $p < 0.001$).

The order in which the treatments were presented had no significant effects on aggressive responses (*H. americana*: $F_{2,375} = 0.79$, $p = 0.454$; *H. cruentata*: $F_{2,102} = 0.63$, $p = 0.54$; *H. occisa*: $F_{2,303} = 1.49$, $p = 0.23$; *H. titia*: $F_{2,306} = 0.07$, $p = 0.93$).

Table 1. Results from paired *t*-tests investigating the relative response to conspecific and heterospecific intruders. Territory holders of the focal species were presented with tethered intruders of their own species and also of the sympatric congener (see §2c for further details). Comparisons that were significant after sequential Bonferroni correction (Rice 1989) are denoted with an asterisk.

focal species	sympatric congener	site	n	t	d.f.	p
H. americana	*H. titia*	AR	16	3.6109	15	0.00257*
H. americana	*H. titia*	CV	33	7.7795	32	<0.0001*
H. americana	*H. cruentata*	EL	17	0.0241	16	0.98107
H. cruentata	*H. occisa*	CT	15	0.714	14	0.48696
H. cruentata	*H. americana*	EL	10	0.8531	9	0.41573
H. occisa	*H. titia*	OT	16	7.3273	15	<0.0001*
H. occisa	*H. titia*	PA	26	4.9414	25	0.00004*
H. occisa	*H. cruentata*	CT	16	0.1437	15	0.88765
H. titia	*H. americana*	AR	14	5.908	13	0.00005*
H. titia	*H. americana*	CV	30	8.2647	29	<0.0001*
H. titia	*H. occisa*	OT	16	6.5646	15	0.00001*
H. titia	*H. occisa*	PA	19	2.7945	18	0.01198

4. DISCUSSION

Where sympatric with *H. titia*, both *H. americana* and *H. occisa* discriminated between conspecific and *H. titia* intruders, responding more aggressively to conspecifics (figure 2). Where sympatric with *H. cruentata*, however, both *H. americana* and *H. occisa* were equally aggressive towards conspecific and *H. cruentata* intruders (figure 2*a,c*). This mirrors Schultz & Switzer's (2001) finding that territorial amberwing dragonflies (*Perithemis tenera*) were likely to pursue heterospecific targets that were most similar in coloration to conspecifics.

We also investigated the effect of wing coloration on heterospecific aggression by using within-site variation in *H. titia* wing spot size. At the two *H. occisa* sites, we found a negative relationship between the proportional size of the hindwing spot of the *H. titia* intruder and the duration of time spent chasing by the *H. occisa* territory holder. That is, *H. titia* individuals with smaller areas of black wing pigmentation received greater aggression from *H. occisa*. We did not find the same pattern at *H. americana* sites, but these sites showed either extremely reduced variation in the size of *H. titia* wing spots (sites AR, CV in April 2008) or low rates of heterospecific chasing (CV in August 2008), diminishing our ability to detect a relationship between heterospecific aggression and wing spot size.

Tynkkynen *et al.* (2004) found that *C. virgo* exhibited greater heterospecific aggression towards *C. splendens* males who resembled *C. virgo* than towards more divergent forms of *C. splendens*. Our results are similar, but the relationship between resemblance and spot size is reversed: *C. splendens* with small wing spots escape aggression by *C. virgo* (a large wing-spotted damselfly), while *H. titia* with large wing spots escape aggression by *H. occisa* (a small wing-spotted damselfly). These results suggest that heterospecific territorial aggression in calopterygid damselflies is influenced by phenotypic similarity as opposed to a simple stimulus-based rule (e.g. attack larger spots more frequently).

Populations of *H. americana* and *H. occisa* that are sympatric with *H. titia* showed reduced aggression towards conspecific male intruders with experimentally blackened wings (which increased their resemblance to *H. titia*), and *H. titia* (always sympatric with a clear-winged congener in this study) showed the opposite response. In contrast, populations of *H. americana*, *H. occisa* and *H. cruentata* that are allopatric from *H. titia* responded with equal aggression towards blackened intruders and clear-winged controls. Sympatric shifts in competitor recognition have also been found in chaffinches (Lynch & Baker 1991) and dendrobatid frogs (Amezquita *et al.* 2006). These putative examples of ACD are unique in that RCD is not a plausible alternative explanation because shifts in recognition occur in potential competitors (same-sex individuals) rather than potential mates (opposite-sex individuals).

Decreased territorial responses to heterospecific coloration were reliably detected at multiple, independent sympatric sites in both *H. americana* and *H. occisa* (figure 3; electronic supplementary material). Parallel results such as these provide strong support for the role of selection over alternative mechanisms (Rundle *et al.* 2000). It is not yet clear, however, whether the geographic pattern that we detected reflects independent evolution of competitor recognition across multiple secondary contact scenarios or a common adaptive reaction norm. Experience has been shown to influence mate recognition in *Enallagma civile* damselflies (Fincke *et al.* 2007). If experience influences competitor recognition in *Hetaerina*, this could be an example of facultative character displacement (Pfennig & Murphy 2002).

Other outstanding questions raised by these results include the following. (i) Is character displacement in the recognition function matched by character displacement in the sexual signal? (ii) Is a reciprocal pattern of character displacement present or is character displacement asymmetric (Cooley *et al.* 2006)? (iii) What is the role of density and frequency dependence in this system? Preliminary evidence indicates that upon repeated visits to sites, neither average spot size nor relative species abundance are necessarily fixed features of a site (C. N. Anderson & G. F. Grether 2007, unpublished
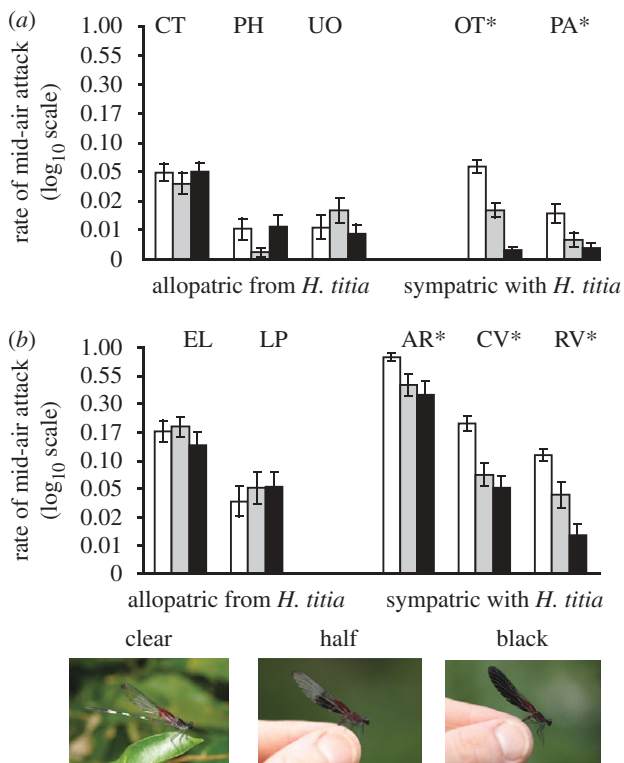
Figure 3. The average attack rate (attacks per second) ±s.e. towards conspecific intruder males with altered wing coloration for tests involving (a) *H. occisa* and (b) *H. americana*. Clear, half and black refer to the hindwing colour treatment (shown in photos). White bar, clear; grey bar, half; black bar, black. Site codes are given above the corresponding bars. Asterisks indicate significant (<0.05) *p*-values based on repeated-measures analyses of variance.
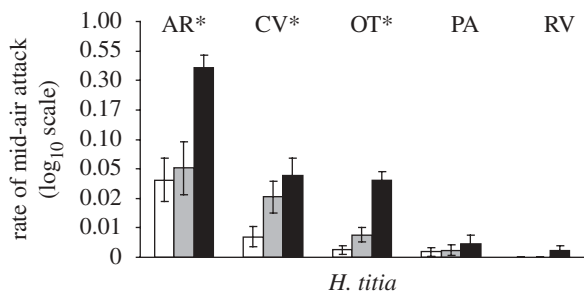

Figure 4. The average attack rate (attacks per second) ±s.e. towards heterospecific intruder males with altered wing coloration for tests involving *H. titia*. Clear, half and black refer to the hindwing colour treatment (see figure 3). White bar, clear; grey bar, half; black bar, black. Site codes are given above the corresponding bars. Asterisks indicate significant (<0.05) *p*-values based on repeated-measures analyses of variance.

data). Nevertheless, the results presented here show that sympatric *H. americana* and *H. occisa* have reduced responses towards intruders with *H. titia*-like wing coloration regardless of whether *H. titia* is numerically dominant or exhibits fully black wing coloration.

In summary, we found evidence for character displacement of competitor recognition in the damselflies *H. americana* and *H. occisa*. Whether these population differences in competitor recognition reflect genetic divergence or adaptive phenotypic plasticity remains to be

determined. Either way, these results demonstrate not only that divergence in wing coloration reduces territorial aggression between sympatric *Hetaerina* species but also that selection has probably shaped these responses. *Hetaerina* can be added to a growing list of examples of ACD (Grether *et al.* 2009).

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