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Sympatric sexual signal divergence among North American *Calopteryx* damselflies is correlated with increased intra- and interspecific male–male aggression

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Abstract Divergence of sexual signals in sympatry can arise as a consequence of (1) interspecific competition for resources, (2) selection against maladaptive hybridization, or (3) as a result of selection to reduce the cost of interspecific aggression; termed agonistic character displacement (ACD). Calopterygid damselflies have emerged as a model system for studying the evolution of divergent sexual signals due to the repeated evolution of sympatric species pairs with fully and partially melanized wings. Damselfly wing patterns function during both courtship and territory defense. However, the relative contributions of natural and sexual selection to phenotypic divergence and enhanced isolation in sympatry remain unclear in many cases. Here, we investigated the hypothesis that interference competition, in the form of increased interspecific male–male aggression, drives the evolution of character displacement in sympatry between two species of North American damselflies, *Calopteryx aequabilis* and *Calopteryx maculata*, that show no evidence of ecological divergence or ongoing hybridization. In paired behavioral trials, we found that interspecific male aggression related to territory defense varied between site, species, and as a function of the relative abundance of con- vs. hetero-specific males. Specifically, we found that large-spotted *C. aequabilis* males received increased intra- and interspecific aggression but that aggression against large-spotted males declined during the middle of the flight season when both species were equally abundant. Based on these results, we suggest that ACD leads to enhanced species recognition, and may be a common outcome of the antagonism between interspecific male–male competition and the countervailing force of intraspecific sexual selection favoring increased wing melanization among territorial damselfly species.

Introduction

Character displacement is defined as the enhancement of phenotypic differences between two species with partially overlapping geographic distributions, where they co-occur (Brown and Wilson 1956). It is generally thought to arise as a consequence of either interspecific competition for resources (ecological character displacement, ECD; Svärdson 1949) or via selection against maladaptive hybridization between partially (i.e., reinforcement; Dobzhansky 1937, 1941) or fully isolated taxa (reproductive character displacement, RCD; Butlin 1987). However, divergence of sexual signals in sympatry may also occur as an indirect result of other forms of selection, such as selection against wasted mating effort and/or interspecific aggression resulting from a "noisy-neighbor" signaling environment (Noor 1999; Pfennig and Pfennig 2009). Sympatric divergence in phenotypic traits arising from this latter form of interference competition, termed agonistic character displacement (ACD; Grether et al. 2009), results from selection acting to reduce the evolutionary cost of interspecific aggressive behaviors (Anderson and Grether 2009; Honkavaara et al. 2011).

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Interference competition leading to agonistic character displacement has only recently begun to receive significant attention in evolutionary studies of secondary sexual signals (Alatalo et al. 1994) and has been most well documented in different species of Calopterygid damselflies (Tynkkynen et al. 2004, 2005, 2006, 2008a, b; Grether et al. 2009; Anderson and Grether 2009; Honkavaara et al.2011). Calopterygid males possess dark, melanized wings, which function in context of both sexual selection and species recognition (Svensson et al. 2004, 2007), and male wing spot size is believed to play an important role in female mate choice (Siva-Jothy 1999; Svensson et al. 2004, 2006, 2007). For example, Tynkkynen et al.(2004) demonstrated that wing spot size in *Calopteryx splendens* was negatively correlated with the relative abundance of *Calopteryx virgo*, where the two species co-occur, suggesting an important role in species recognition. They also found elevated levels of interspecific aggression in sympatry and differences between these two species in their ability to hold territories, concluding that strong negative selection on wing spot size underlies character displacement in this system (Tynkkynen et al. 2004, 2005). Additional work has subsequently found evidence for reciprocal hybridization between these two damselfly species (Tynkkynen et al. 2008a), and suggested that male mate discrimination against heterospecific females depends on both male ornament size and differences in reproductive strategy (Tynkkynen et al. 2009). Indeed, empirical data indicate that local processes are important for shaping the evolutionary dynamics of species interactions among European *Calopteryx* species (Svensson et al. 2010) and, furthermore, that these local dynamics are most heavily influenced by the presence or absence of sympatric sexual competitors (Svensson 2012; Wellenreuther et al. 2010, 2012). Taken together, these results suggest that interspecific aggression may drive divergence in both agonistic signals and competitor recognition functions among sympatric taxa (Anderson and Grether 2009; Grether et al. 2009).

Despite the strong evidence for interspecific aggression among sympatric, territorial damselfly species, it is clear that other forms of selection also heavily influence divergence in sexual signals. For example, both ecological selection for thermoregulation (Outomuro and Ocharan 2011) and the response of the immune system to parasite load (Siva-Jothy 2000; Córdoba-Aguilar 2002) can influence the degree of wing melanization. In addition, predation has been shown to select for increased immune function in male damselflies (Rantala et al. 2011) and be more intense on territorial individuals (Toivanen et al. 2009). This is important because predation risk varies in relation to the degree of wing melanization among sympatric populations of *C. splendens* and *C. virgo* (Svensson and Friberg 2007) and, as a consequence, may indirectly influence the evolution of male secondary traits. Intraspecific selection on secondary sexual

traits, therefore, may sometimes act in opposition to selection generated by interspecific male–male interactions upon secondary contact (Kuitunen et al. 2011). Finally, it is unclear what role selection against maladaptive hybridization (i.e., reinforcement) plays relative to the direction of selection generated by interference competition among damselfly populations (Tynkkynen et al. 2008a). More generally, we lack good comparative evidence from other sympatric comparisons of territorial damselflies to evaluate how commonly agonistic behaviors contribute to trait divergence in phenotypes also used in sexual signaling.

Although disentangling the various forms, directions, and relative contributions of different types of selection acting on male secondary sexual traits is beyond the scope of any single study (but see Svensson et al. 2006), investigations of agonistic behaviors in territorial species that co-occur over part of their range can inform our understanding of how ubiquitous interference competition may be. In addition, examples of sympatric character displacement for which selection against hybridization can be excluded as a potential cause may provide more compelling evidence for the importance of interspecific aggression in the evolution of secondary sexual signals. For example, male Ebony Jewelwing (*Calopteryx maculata*) damselflies have completely melanic wing patterns, which show little geographic variation. In contrast, River Jewelwing (*Calopteryx aequabilis*) males display a pattern of distal wing melanization that is geographically variable (Waage 1975). Classic work on these two species conclusively demonstrated that the degree of male wing pattern melanization varies with the relative frequency of heterospecifics and is strongly displaced in sympatry (Waage 1975, 1979). Furthermore, Waage (1979) also found evidence that sympatric Ebony and River Jewelwing males display enhanced mate discrimination between con- and heterospecific females, suggesting an important role for selection against interspecific matings. Although these findings are consistent with broad sense definitions of reinforcement (sensu Servedio and Noor 2003), a more recent genetic evaluation found no evidence for hybridization or introgression (Mullen and Andrés 2007), suggesting that selection acting to reduce interference between the mate recognition signals of these non-hybridizing species (Noor 1999; Pfennig and Pfennig 2009) may best explain the observed pattern of reproductive character displacement in melanic wing phenotypes.

Here, we return to this system to investigate the hypothesis that interspecific aggression arising as a result of incomplete species recognition in a "noisy-neighbor" signaling environment (Otte 1989; Noor 1999) underlies the pattern of character displacement observed in male wing pattern coloration. Overall, we predict that the Ebony Jewelwing will demonstrate increased aggression towards River Jewelwings with larger black spots, as these heterospecifics will bear a stronger resemblance (De Marchi 1990; Tynkkynen et al.

2004). Furthermore, we predict that interspecific aggression will be correlated with the relative abundance of each species and will be less intense when males of each species are equally abundant because frequent interspecific encounters will select for increased species discrimination, thus reducing interspecific aggression. To test this prediction, we used behavioral trials and sampled repeatedly across a single breeding season to control for difference in aggression arising as a result of temporal variation in abundance. In addition, we presented males of each species with heterospecific males that had either large or small melanized wing spots to test the prediction that increased melanization, and hence increased signal similarity, would elicit elevated levels of interspecific aggression as measured by reaction distance. Our results suggest that agonistic character displacement (ACD) may be a common outcome of the antagonism between interspecific male–male competition and the countervailing force of intra-specific sexual selection favoring increased wing melanization among territorial damselfly species.

Materials and methods

Study organisms

In the damselfly species *C. aequabilis* and *C. maculata* (Order: Odonata; Family: Calopterygidae), males compete with each other for territories containing egg-laying sites, and females mate with males occupying the highest quality territories (Waage 1975). *C. maculata* (the Ebony Jewelwing) has black wings and is found from the south east provinces of Canada down to southeast United States and as far west as Kansas. *C. aequabilis* (the River Jewelwing) has clear wings with varying degrees of melanization and appears in most of northern and western states of the United States and southern parts of Canada. Although both species occupy territories along streams and small rivers, *C. maculata* has a wide habitat tolerance whereas *C. aequabilis* has more habitat specificity and tends to be found only in wide streams with fast flow.

Field sites

The experiment was performed over two summers at two field sites along Fall Creek in Western New York where the species were known to co-occur (Mullen and Andrés 2007). The two sites were at Groton Avenue Park in Freeville, NY (42°52′N, 76°35′W) and Route 13 in Ithaca, New York (42°47′N, 76°42′W), and they resembled each other with respect to plant diversity, stream cover, and water temperature. These two sites are separated by 4.75 km and previous work suggests that both *C. aequabilis* and *C. maculata* are unlikely to travel more than 750 m (Pither and Taylor 1998; Jonsen and Taylor 2000). Therefore, we were confident that these field sites represented geographically separate but similar populations, which differ primarily in the relative abundance of both species.

Relative abundance

Relative abundance data for *C. aequabilis* and *C. maculata* were collected on three dates (from the middle of June to the end of July) that represented the early, middle, and late parts of the season. Sampling was done only on days with optimal weather and maximum activity (warm temperatures and minimal wind) to minimize differences due to variation in weather conditions. On each sampling date, we caught and recorded species and spot size of all *Calopteryx* individuals along a haphazardly selected a 100-m section of the stream within the larger stream region where density was the greatest. These individuals were also marked on the abdomen and eventually used as focal individuals later in the reaction distance portion of the experiment. Data from 2010 and 2011 were combined to increase sample size because no differences were found in the relative abundances of *C. aequabilis* and *C. maculata* between years at each site for any given time period (Fisher's exact test, $p > 0.5$ for all tests). We then performed a logistic regression on the merged data to test for differences in the relative abundance using species identity tested against time period (early, middle, late), site (Groton, Route 13), and the interaction of site and time period.

Spot size and wing length

We took several measurements of the hind wings of each individual with a digital caliper to the nearest 0.01 mm. Repeatability of caliper measurements was high, as there was a strong correlation between two length measurements taken for a subset of individuals to confirm accuracy ($r^2 = 0.98$; $F_{1,22} = 981.77$, $p < 0.0001$). We measured the length of the left and right hind wing (from where it attached to the body to the tip of the wing) and averaged the two values for subsequent analyses. The area of the wing spot was estimated by measuring the length of the spot from the tip of the wing to the area where the melanization no longer appeared on the wing; we also recorded the width at the midpoint of the spot. T tests were used to compare spot sizes and wing lengths across sites.

Reaction distance and aggression

We tested the aggression of *C. maculata* and *C. aequabilis* by using tethered live damselflies as "intruders" to be moved toward territorial males. Although dead or model damselflies would have been easier to manipulate, and have been successfully used in similar damselfly experiments, they did not induce an aggressive response in this system (unlike

Tynkkynen et al. 2004). Intruders of both species were caught at least 500 m away from where the presentations were done.

One day before presentations, we caught male *C. aequabilis* and *C. maculata* in a 100-m area to use as territorial residents. These were marked with colored bands (green, yellow, white, red, blue, and silver) on the individual's abdomen using DecoColor^TM Opaque paint markers. Once they were marked and wing measurements were made, we released them back into the area where they were taken from. If one of these marked males was seen in the same 1-m area as the day before and observed portraying territorial characteristics, this male was determined to be territorial (Pajunen 1966).

All presentations were done by the same observer, and each presentation involved an intruder attached to a fishing line with a piece of tape around the abdomen to allow wing movement. Presentations began by starting the intruder approximately 2 m away from the territorial male, based on a pre-measured field marker, and moving the fishing pole (with the attached intruder) slowly towards the territorial male. We measured the reaction distance by measuring the distance from the intruder to the territorial male when it left its perch, and we also recorded whether the resident's aggression was an approach (no contact) or an attack (direct contact; Tynkkynen et al. 2004). The experiment was performed on 59 total territorial males (13 *C. aequabilis*, 14 *C. maculata* at Groton Ave.; 16 *C. aequabilis*, 16 *C. maculata* at Route 13) where each territorial male faced sequential presentations of three intruder males (small-spotted *C. aequabilis*, large-spotted *C. aequabilis*, and *C. maculata*) in random order. *C. aequabilis* intruders were chosen such that those designated as large-spotted individuals were significantly larger than the small-spotted ones in each trial (paired $t = 16.02$, $df = 19$, $p < 0.0001$) despite having similar wing lengths (paired $t = 2.02$, $df = 19$, $p = 0.06$). Reaction distance, as a dependent variable, was analyzed using a four-way ANOVA using intruder phenotype, territorial species, site, and time as factors, along with the associated interaction terms; non-significant terms were removed one at a time until only significant explanatory variables remained in the model (see Table S1 for all terms). Additionally, we performed a logistic regression on the attack versus approach data to determine whether more aggressive damselflies (i.e., those that made direct contact) tended to have farther reaction distances.

Results

Relative abundance, spot size, and wing length

The results of our logistic regression analysis indicate that the abundance of *C. maculata* relative to *C. aequabilis* changed significantly from late June to July, differed between sites, and that the variation in the relative abundance of each species

among time periods was different at each site (Table 1). Specifically, we found that *C. maculata* were far more prevalent than *C. aequabilis* at the Groton site throughout the entire season, but that, in contrast, both species were equally abundant at Route 13 during the middle of the season (Table 2). There were no notable phenotypic differences between the individuals at both sites, as *C. aequabilis* had similar spot sizes ($t = 1.36$, $df = 130$, $p = 0.18$) and wing lengths ($t = 1.57$, $df = 130$, $p = 0.12$) at both sites, and the wing lengths of *C. maculata* were not significantly different across sites ($t = 0.89$, $df = 284$, $p = 0.38$).

Reaction distance and aggression

Intruder phenotype, the species identity of the territorial male, site location, and time period were all significant predictors of male aggression and reaction distance in our ANOVA (Table 3). In addition, the interaction between intruder phenotype and territorial male was highly significant, indicating that the two different species react differently to the species identity and wing spot size of the intruder. We also found a significant interaction between intruder phenotype and site, indicating that different types of intruders are treated differently between sites. For example, we found that *C. aequabilis* males at both sites modulated their aggression based on the identity of the intruder (Fig. 1a), and showed low levels of aggression towards small-spotted *C. aequabilis* and *C. maculata* relative to large-spotted *C. aequabilis*. In contrast, *C. maculata* males reacted differently the three intruder types at each site (Fig. 1b). At the Groton Ave. site, *C. maculata* reacted similarly to conspecifics and the large-spotted *C. aequabilis*. However, *C. maculata* were 71.7 % less aggressive towards the small-spotted versus large-spotted *C. aequabilis*. In comparison, at the Route 13 site, *C. maculata* reacted to the large-spotted *C. aequabilis*, small-spotted *C. aequabilis*, and *C. maculata* with different amounts of aggression, and were 45.5 % less aggressive towards large-spotted *C. aequabilis* and 71.6 % less aggressive towards small-spotted *C. aequabilis* relative to conspecifics. Importantly, differences in aggression were not the result of territorial male and site interactions or, surprisingly, a function of interactions between the intruder phenotype, territorial species, and site. Instead, we found that

Table 1 Results of logistic regression model regarding the proportion of *C. maculata* (relative to *C. aequabilis*) based on the time period and site ($\chi^2 = 19.8082$, $df = 3$, $p = 0.0002$)

Source	df	χ^2	p value
Time period (early, middle, late)	1	9.748	0.0018
Site (Groton, Rt. 13)	1	5.680	0.0172
Site × time period	1	6.849	0.0089

Table 2 The variation in relative abundance of *C. aequabilis* and *C. maculata* males at both field sites throughout the summer based on sampling dates

Sampling period	Site	*C. aequabilis*	*C. maculata*	Difference in relative abundance?
Early	Rt. 13	17	36	Yes (χ^2=6.97, df=1, p=0.008)
(June 1–20)	Groton	7	17	Yes (χ^2=4.30, df=1, p=0.038)
Middle	Rt. 13	29	29	No (χ^2=0.00, df=1, p=1.000)
(June 25–July 4)	Groton	9	34	Yes (χ^2=15.49, df=1, p<0.001)
Late	Rt. 13	16	81	Yes (χ^2=47.60, df=1, p<0.001)
(July 7–31)	Groton	15	101	Yes (χ^2=71.47, df=1, p<0.001)

resident *C. maculata* and *C. aequabilis* differ in their reaction (i.e., level of aggression) to different types of intruders based on the sampling period (i.e., time of year).

Consistent with this result, we found that at the Groton Ave. site, where *C. maculata* was more abundant than *C. aequabilis* throughout the entire season (Tables 1 and 2), *C. maculata* demonstrated more aggression as evidenced by having a higher proportion of interactions that resulted in direct contact (attacks; Table 4; Fisher's exact test, p<0.0001). In contrast, at the Route 13 site, where both species were equally abundant in the middle of the season, *C. aequabilis* males had a higher proportion of attacks versus approaches (Fisher's exact test, p=0.0187). When analyzing species across sites, we found that *C. maculata* were more aggressive at the Groton Ave. site relative to the Route 13 site (Fisher's exact test, p<0.0001), whereas *C. aequabilis* were equally aggressive at both sites (Fisher's exact test, p=0.6880). However, our four-way ANOVA interaction term including intruder, territorial species, time, and site was not significant. Finally, our logistic regression analyses revealed that more aggressive damselflies (i.e., those that made direct contact) tended to respond from longer distances in both species (*C. maculata*—n = 164 overall, model pseudo-r^2=0.36, p=0.009; *C. aequabilis*—n = 144 overall, model pseudo-r^2=0.33, p= 0.018), thus confirming the positive relationship between the intensity of aggression and reaction distance.

Discussion

Our study tested the hypothesis that sympatric divergence in sexual signals between Ebony (*C. maculata*) and River (*C.*

aequabilis) Jewelwing damselflies arises as a consequence of selection acting on traits that influence interspecific aggressive behaviors (Anderson and Grether 2009), also known as agonistic character displacement (ACD). In paired behavioral trials, we found that interspecific male aggression varied between site, species, and as a function of the relative abundance of con- versus hetero-specific males. Specifically, we found that aggression by male *C. maculata* towards heterospecific males differed between sites and was highest at the Groton Ave. site where they were numerically more abundant than *C. aequabilis* throughout the entire season. In contrast, male *C. aequabilis* displayed consistently high levels of aggression towards large-spotted *C. aequabilis* males relative to either small spotted conspecifics or heterospecific *C. maculata* males, at both sites.

Our analyses revealed no significant interactive effect between the territorial males' species identity, intruder phenotype, and site on aggression. This suggests that site-specific differences in the way that different territorial males treat different intruder phenotypes arise as a function of variation in the relative abundance of each species at different time periods, and we hypothesize that this effect is swamped out when we lump data across the entire summer (i.e., when we include a four-way term with time in our model). Indeed, the results of our logistic regression analysis support this hypothesis and indicate that there are different seasonal species frequency dynamics at the two sites (Tables 1 and 2). This is important because reproductive success for male Calopterygid damselflies is dependent on territory maintenance and defense (Waage 1973; Corbet 1980), and may be heavily influenced by seasonal fluctuations in the abundance of heterospecific males.

Table 3 Results of four-way ANOVA on reaction distance based on the intruder's phenotype, the territorial species, site, and time period (non-significant factors have been removed)

Source	df	Sum of squares	F Ratio	p value
Intruder phenotype (SS AEQ, LS AEQ, MAC)	2	146,783.90	78.9805	<0.0001
Territorial species (AEQ, MAC)	1	4,159.59	4.4763	0.0352
Site (Groton, Rt. 13)	1	9,128.71	9.8238	0.0019
Time period (early, middle, late)	2	10,354.67	5.5716	0.0042
Intruder × territorial	2	119,940.94	64.5370	<0.0001
Intruder × site	2	30,679.78	16.5080	<0.0001
Intruder × territorial × time	4	15,529.32	4.1780	0.0026
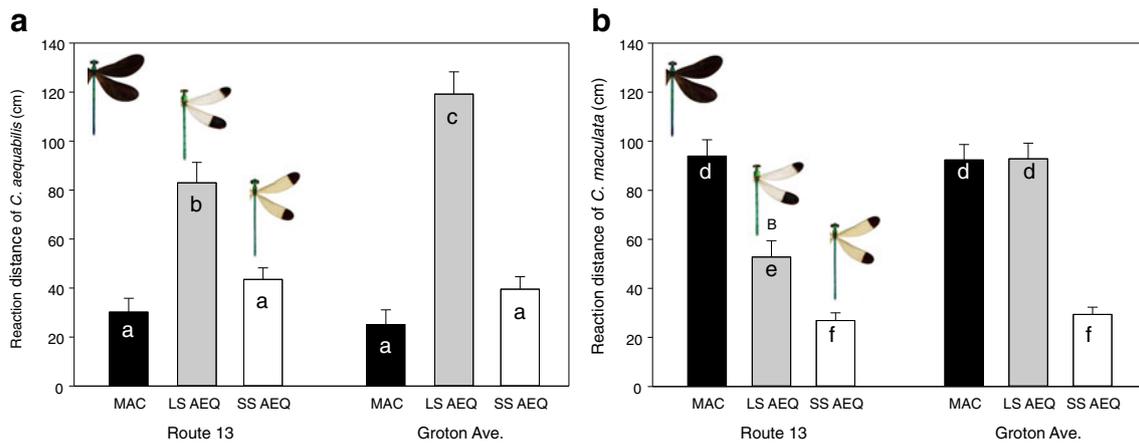
Fig. 1 The reaction distance (cm) of territorial males towards con- and heterospecific individuals. For each territorial species, significant differences both within and between sites are denoted by different letters (Tukey's post hoc test, $p < 0.05$; see also Table 3). a *C. aequabilis* tested against *C. maculata* (*MAC*) and small-spotted (*SS AEQ*) and large-spotted *C. aequabilis* (*LS AEQ*) at both sites. b *C. maculata* tested against *C. maculata* (*MAC*) and small-spotted (*SS AEQ*) and large-spotted *C. aequabilis* (*LS AEQ*) at both sites

It is important to note, however, that different time periods mark changes in the absolute and relative abundances of both species. Thus, future studies must be done to disentangle the effects of conspecific and heterospecific densities, both of which many affect competition (Noriyuki et al. 2012; Friberg et al. 2013).

The dependence of male reproductive success on territory defense suggests that any trait that increases the intensity or frequency of aggressive interactions is likely to be under strong selection. Jewelwing damselfly males defend territories along streams, typically within 2 m of female oviposition sites (Waage 1973), and Calopterygid males of other species with larger wing spots or increased wing pigmentation are more likely to be territorial than conspecific males with less pigmentation (Grether 1996a, b; Siva-Jothy 1999; Córdoba-Aguilar 2002). Females typically prefer territorial males (Waage 1973; Plaistow and Siva-Jothy 1996; Córdoba-Aguilar 2002; Serrano-Meneses et al. 2007) and, as a consequence, previous work suggests that territorial males experience higher mating success (Plaistow and Siva-Jothy 1996; Suhonen et al. 2008). However, territory holding ability appears to be primarily a function of body condition (Marden and Waage 1990; Marden and Rollins 1994; Plaistow and

Siva-Jothy 1996; Koskimaki et al. 2004; Contreras-Garduño et al. 2006), with larger males (Beck and Pruett-Jones 2002) or males with higher levels of body fat and/or lower parasite loads better able to defend territories (Koskimaki et al. 2004; 2009).

In this study, we found that large-spotted male *C. aequabilis* experience increased levels of both intra- and interspecific aggression. We hypothesize that larger wing spots result in mistaken species recognition by *C. maculata* and, hence, increased interspecific aggression against large-spotted *C. aequabilis* males. In addition, as noted above, larger spotted males are more likely to be territorial and, therefore, may also receive increased intraspecific male aggression from conspecific males related to territory defense. Because damselfly territorial contests are energetic wars of attrition (Marden and Waage 1990; Cordero 1999; Contreras-Garduño et al. 2009), the increased intensity of male–male aggressive interactions in sympatry could lead to a reduction in the body condition of large-spotted *C. aequabilis* males and, hence, erode their ability to maintain territories (sensu Tynkkynen et al. 2005), resulting in character displacement.

Although the reduction in wing spot size among male *C. aequabilis*, where it co-occurs with *C. maculata*, has previously been considered a classic example of reinforcement (Waage 1975, 1979), and there is clear evidence for reinforced female mate preferences based on wing color among European *Calopteryx* (Svensson et al. 2007, 2010), a previous study by Mullen and Andrés (2007) found no evidence of hybridization between these two North American species. Furthermore, levels of sequence divergence between these species suggest they diverged in the early Miocene (10–15 mya), are fully isolated, and have only experienced sympatry due to secondary contact following recent postglacial range expansion (~12 kya). These findings are inconsistent with the narrow sense definition of reinforcement (i.e., selection against hybrids) and, in light of the

Table 4 Number of attacks (direct contact) vs. approaches (chasing without contact) based on the territorial species and site

Territorial species	Attack	Approach	% Attack
Groton			
C. maculata	25	52	32.50
C. aequabilis	4	62	6.06
Route 13			
C. maculata	4	83	4.60
C. aequabilis	13	65	16.67

current results, suggests that sympatric divergence in sexual signals in this system arose as a by-product of intense interspecific male–male aggression (Tynkkynen et al. 2004, 2006) or potentially as a result of selection against wasting mating effort (e.g., Noor 1999; Pfennig and Pfennig 2009). Although both mechanisms may operate in this system, our results are consistent with several recent studies (Tynkkynen et al. 2004, 2005; Anderson and Grether 2009; Honkavaara et al. 2011) that suggest agonistic behavior plays an important role in shaping the morphology of male sexual ornaments in sympatry.

It is less clear, however, what role female preferences may play in shaping male sexual ornaments in this system. Previous work suggests that *Calopteryx* females base mate choice decisions on both male territoriality and wing pigmentation (Siva-Jothy 1999; Córdoba-Aguilar 2002). Although, in many cases, *Calopteryx* females have been shown to preferentially mate with territorial males and to prefer males with large wing spots or increased wing pigmentation (Svensson et al. 2006, 2007), recent work by Kuitunen et al. (2011) found that *C. splendens* females do not display consistent mating bias towards large-spotted male conspecifics. While this result is at first surprising, it is possible that interference competition from sympatric *C. virgo* males may influence female mate preference relative to females in allopatry. This is an intriguing hypothesis, which, if true, suggests that intraspecific, intersexual selection, as manifested by female mate choice preferences, may at times conflict with intrasexual selection (i.e., interspecific male–male competition) between sympatric damselfly species. If so, contrasting selective forces in the sympatric versus allopatric areas could ultimately reduce gene flow among intraspecific populations and potentially initiate speciation (e.g., see Hoskin et al. 2005; Pfennig and Ryan 2006; Pfennig and Rice 2007; Pfennig and Pfennig 2009; Rice and Pfennig 2010). Experimental tests of this hypothesis are currently underway in the North American system to examine whether female *C. aequabilis* mating preferences have diverged between areas of sympatry and allopatry with *C. maculata*.

In conclusion, although teasing apart the relative contributions of individual forces to the evolution of male sexual signals with require additional field studies, the results of this, and other recent studies (Tynkkynen et al. 2004, 2005, 2006; Grether et al. 2009; Anderson and Grether 2010; Honkavaara et al. 2011; Kuitunen et al. 2011), suggest that ACD may be a common outcome of the antagonism between interspecific male–male competition and the countervailing force of intra-specific sexual selection favoring increased wing melanization among territorial damselfly species.

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