

Durham Research Online

Deposited in DRO:

19 July 2018

Version of attached file:

Accepted Version

Peer-review status of attached file:

Peer-reviewed

Citation for published item:

Drury, J.P. and Grether, G.F. (2014) 'Interspecific aggression, not interspecific mating, drives character displacement in the wing coloration of male rubyspot damselflies (Hetaerina).', *Proceedings of the Royal Society series B : biological sciences.*, 281 (1796). p. 20141737.

Further information on publisher's website:

<https://doi.org/10.1098/rspb.2014.1737>

Publisher's copyright statement:

Additional information:

Use policy

The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:

- a full bibliographic reference is made to the original source
- a [link](#) is made to the metadata record in DRO
- the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the [full DRO policy](#) for further details.

1 **Interspecific aggression, not interspecific mating, drives character displacement in the wing**
2 **coloration of male rubyspot damselflies (*Hetaerina*)**

3

4 Drury, Jonathan P.¹ & Grether, Gregory F.¹

5

6 ¹University of California, Los Angeles

7 Department of Ecology & Evolutionary Biology

8 612 Charles E. Young Dr. S.

9 Los Angeles, CA 90095

10 phone: (310) 825-3128

11

12 Authors for correspondence: Jonathan Drury (druryj@ucla.edu) & Gregory Grether

13 (ggrether@ucla.edu)

14

15 **Keywords**

16 *agonistic character displacement, competitor recognition, mate recognition, interspecific*

17 *aggression, species recognition,*

18

19 Word count (including title, summary, keywords, text, figure legends and references): 6400

20

21 **Summary**

22 Traits that mediate intraspecific social interactions may overlap in closely related
23 sympatric species, resulting in costly between-species interactions. Such interactions have
24 principally interested investigators studying the evolution of reproductive isolation via
25 reproductive character displacement (RCD) or reinforcement, yet in addition to reproductive
26 interference, interspecific trait overlap can lead to costly between-species aggression. Previous
27 research on rubyspot damselflies (*Hetaerina* spp.) demonstrated that sympatric shifts in male
28 wing colour patterns and competitor recognition reduce interspecific aggression, supporting the
29 hypothesis that agonistic character displacement (ACD) drove trait shifts. However, a recent
30 theoretical model shows that RCD overshadows ACD if the same male trait is used for both
31 female mate recognition and male competitor recognition. To determine if female mate
32 recognition is based on male wing coloration in *Hetaerina*, we conducted a phenotype
33 manipulation experiment. Compared to control males, male *H. americana* with wings
34 manipulated to resemble a sympatric congener (*H. titia*) suffered no reduction in mating success.
35 Thus, female mate recognition is not based on species differences in male wing coloration.
36 Experimental males did, however, experience higher interspecific fighting rates and reduced
37 survival compared to controls. These results greatly strengthen the case for ACD and highlight
38 the mechanistic distinction between ACD and RCD.

39

40 **1. Introduction**

41 When closely related species come into secondary contact, they may overlap in traits
42 used as intraspecific social signals, resulting in costly interactions between species. Evolutionary
43 biologists have focused primarily on the ways in which selection acts to reduce the occurrence of

44 costly reproductive interactions between heterospecific males and females in the context of
45 reproductive character displacement (RCD) and reinforcement [1–7]. Interference competition
46 between species, which in animals usually takes the form of aggressive interactions, is also very
47 common [8], yet agonistic character displacement (ACD), a process whereby natural selection
48 acts on traits that mediate the occurrence or outcome of interspecific aggression, remains
49 relatively understudied [9,10]. While RCD and ACD can result in the same geographic patterns,
50 the processes are conceptually distinct, because interspecific interference competition need not
51 be related to competition for mates [10], and the dynamics of trait evolution can proceed quite
52 differently [9,10]. As such, studies of selection on traits that mediate interspecific social
53 interactions should distinguish between these two processes when drawing conclusions about the
54 evolutionary history of such traits.

55 Many phenotypic traits function as signals in both mating and competitive contexts [11]
56 (see Table 1 in [12]), and, in some cases, the same character displacement patterns (e.g.,
57 sympatric shifts in phenotypes) have been attributed to both ACD and RCD. In the best known
58 example, male pied flycatchers (*Ficedula hypoleuca*) have black dorsal plumage in allopatry, but
59 in sympatry with the dominant collared flycatcher (*Ficedula albicollis*), most male pied
60 flycatchers have dull, brown plumage, which resembles female collared flycatchers [13] and
61 reduces territorial aggression from male collared flycatchers [14–16]. The same plumage shift
62 also reduces the rate of cross-species mating and hybridization because female pied flycatchers
63 prefer males with brown plumage in sympatry, which represents a reversal of the preference for
64 black males in allopatry [17]. In another well-studied example, male *Calopteryx splendens*
65 damselflies have blue-black wing spots that are larger in allopatry than in sympatry with *C.*
66 *virgo*, which have fully blue-black wings [18,19]. Moreover, *C. virgo* males are more aggressive

67 to *C. splendens* males with relatively larger wing spots in sympatry, which consequently affects
68 male fitness [18,20,21], yet female mate recognition is also influenced by male wing coloration
69 and shifts in sympatry in a manner consistent with RCD [22,23].

70 In a recent theoretical study, Okamoto & Grether [15] set out to understand whether ACD
71 and RCD can act synergistically to drive evolutionary divergence, or whether one process has
72 priority over the other. They constructed an individual-based theoretical model based on
73 territorial damselflies to explore how RCD and ACD interact when female mate recognition and
74 male competitor recognition are based on the same male trait. The male trait closely tracked the
75 evolution of the mate recognition function, regardless of the relative strength of selection against
76 interspecific mating and interspecific fighting. Even in the absence of selection against cross-
77 species mating, a trait on which female mate recognition is based cannot diverge through ACD in
78 this model. The basic reason is that mutations that reduce interspecific aggression by causing a
79 male's phenotype to deviate from the mean of the other species also reduce his ability to attract
80 conspecific females, and thus have a net negative effect on fitness. Okamoto & Grether's [15]
81 model also showed that sympatric shifts in competitor recognition, which previously were
82 thought to constitute *de facto* evidence for ACD, can arise as a byproduct of trait divergence
83 caused by RCD. This is because males still need to recognize conspecific males as competitors,
84 as the trait diverges through RCD. In short, RCD completely dominates ACD in this model.
85 Therefore, to conclude that ACD is responsible for an observed character displacement pattern,
86 RCD needs to be ruled out as an alternative explanation.

87 Previous research on two species pairs of rubyspot damselflies (*Hetaerina* spp.) showed
88 that male competitor recognition is based on wing coloration [24,25] and that competitor
89 recognition and male wing coloration in these species pairs diverge in sympatric populations

90 [24,26]. These results are consistent with the hypothesis that ACD has acted in these species
91 pairs. Based on Okamoto & Grether's [12] findings, however, these trait shifts cannot be taken as
92 compelling evidence for ACD unless females do not use male wing coloration for species
93 recognition. While attempts to detect female mate choice based on male coloration within
94 species of *Hetaerina* have yielded no such evidence [27,28], whether females use male
95 coloration for species discrimination is unknown.

96 Here we test for effects of male wing coloration on female mate recognition in *H.*
97 *americana* in a population sympatric with *H. titia*, which is one of the species pairs in which
98 sympatric divergence in male coloration and competitor recognition has been detected. Male *H.*
99 *americana* have large basal red wing spots and otherwise clear wings (Fig. 1a) while male *H.*
100 *titia* have smaller basal red wing spots and variable amounts of black wing pigmentation (Fig.
101 1b, electronic supplementary material, Figure S1). To test whether female *H. americana* use the
102 species difference in male wing coloration to avoid mating with heterospecific males, we
103 conducted a field experiment in which a portion of *H. americana* males in the study area were
104 manipulated to resemble *H. titia* males with black ink. We then tracked naturally occurring
105 mating events, territorial fights, changes in territory ownership, and survival on a continuous
106 basis for 5 weeks.

107

108 **2. Methods**

109 *(a) Study site and species*

110 We carried out this study on two transects (~100 m each) marked at 1 m intervals along
111 the Medina River in Castroville, TX (29.371797°, -98.896444°; 29.374733°, -98.896769°) from
112 May 23rd to June 23rd 2013. To minimize dispersal, the study transects were located such that

113 long pools (> 100 m), which are not suitable as breeding habitat, were located both up and
114 downstream. Every individual American rubyspot (*Hetaerina americana*) and smoky rubyspot
115 (*H. titia*) damselfly encountered along these transects was captured with an aerial net and marked
116 on its abdomen with a unique combination of DecoColor paint pens [24,29]. *Hetaerina* perch
117 with their wings folded above their bodies, so abdomen marks usually are clearly visible to
118 observers.

119

120 *(b) Experimental wing manipulation*

121 When we captured mature *H. americana* males, we assigned them to one of three
122 treatments: 1) unmanipulated control (Fig. 1a), 2) clear control: clear ink on the outer surface of
123 the hindwings from the base to halfway between the nodus and the tip using a Prismacolor
124 marker (clear, PM-121, Fig. 1c), or 3) blackened: black ink on the same part of the hindwings
125 (black, PM-98, Fig. 1d), following the protocol of Anderson & Grether [25] (see [30] for a
126 similar approach to phenotype manipulation). To maximize the statistical power to detect effects
127 of the experimental treatment, half of the males were assigned to the blackened treatment and
128 25% were assigned to each of the control groups. We restricted the experimental blackening to
129 hindwings to prevent males' wings from sticking together, which is possible if fore- and
130 hindwings are both treated with ink (G.F. Grether pers. obs). Although some *Hetaerina titia*
131 males have extensive black pigmentation on their forewings, many do not (Calvert 1908;
132 Johnson 1961; also see electronic supplementary material, Fig. S1), so our experimental
133 manipulation was biologically realistic. Throughout the study, mature *H. americana* males were
134 marked and assigned to a treatment group as they appeared or reached maturity on the transects

135 (mature males have brilliant red forewing spots while immature males have pink to light red
136 forewing spots [31]).

137

138 *(c) Behavioural observations*

139 During each day of the study, 3-5 observers, typically 4, continuously walked along the
140 transects from ~9.00 to 18.00 h, systematically recording the location to the nearest 0.1 m and ID
141 of each individual encountered, with priority given to recording tandem (mating) pairs and
142 fighting individuals. We strived to record all matings, which is quite feasible because tandem
143 pairs are conspicuous and stay together for several minutes.

144 *Hetaerina* mating sequences do not include courtship, instead they begin when a male
145 clasps a female's prothorax (intersternite), at which point the pair is in tandem [32]. From here,
146 the tandem pair either breaks up without proceeding, which we considered a failed mating
147 attempt, or continues on to form the characteristic copulatory wheel of odonates. In *H.*
148 *americana*, after the copulatory wheel breaks, the tandem pair exhibits a jerking motion before
149 the female submerges to oviposit in underwater vegetation [33]. When we encountered a mating
150 pair, we recorded the IDs of both individuals and followed them until the mating was either
151 successful (i.e., we saw a copulatory wheel, jerking, or submersion) or the tandem broke. When
152 possible, we recorded the entire length of time the pair was in the copulatory wheel. We also
153 recorded instances where we observed a male pursue and fail to grasp a female and considered
154 these to be failed mating attempts.

155 When an observer witnessed a fight, the location, species involved, ID of individuals (if
156 marked), and escalation level were recorded; we considered two-way circle chases or back-and-
157 forth chases [27,34] to be "escalated" in subsequent analyses.

158

159 (d) *Female mating analyses*

160 Females may make post-copulatory decisions that bias paternity, since subsequent mates
161 can remove previous mates' sperm from females' sperm storage organs [45,46]. To test for this
162 possibility, we analyzed (1) the treatments of females' first and last mates during each day and
163 (2) whether males' treatments influenced whether females remated or the treatment of
164 subsequent mates. Nearly all females' mating bouts (N consecutive days observed in a mated
165 pair) lasted for three days or fewer, so to test for the possibility that sperm removal influenced
166 male mating success, we analyzed female remating (1) within each day and (2) across a three-
167 day window.

168

169 (e) *Data analyses*

170 In several analyses, we partitioned the reproductive career of individual males into
171 territorial and non-territorial episodes in order to distinguish between the effects of male-male
172 interactions and male-female interactions [27]. The territorial status of a given male on a given
173 day was assessed based only on the male's resighting and fighting record and without knowledge
174 of his treatment group or mating success. We considered males to be holding a territory if they
175 were resighted consistently on a low perch near the bank of the river within a 3 m radius for at
176 least two consecutive days [24]. Additionally, we took fighting and resights in the same area near
177 the stream over a period of several hours to be evidence that a male was holding a territory.
178 To analyze fighting rates, we took three approaches: (1) treating all recorded fights between the
179 same two males as a single fight (as in [25]), (2) treating fights between the same two males on N
180 different days as N different fights (i.e., one fight recorded per dyad per day), and (3) treating all

181 fights as unique whether they were between the same or different males (i.e., all fights recorded
182 per dyad per day). *H. titia* male densities were not consistent along the entire length of the
183 transect. Because the wing blackening treatment was only expected to affect males that
184 interacted with *H. titia* males, we restricted some analyses to males that were observed within
185 close proximity (≤ 4 m), of a *H. titia* territory holder. The 4 m criterion was chosen, *a priori*,
186 based on the observation that the reaction distance of territory holders to conspecific male
187 intruders is ≤ 2 m and that adjacent territories are typically ≤ 2 m apart, as reported previously
188 [25].

189 Because the opportunity for males to fight and mate depended on the number of days
190 they were present in the study, we analyzed the data using count models with exposure terms of
191 the logarithm of the number of days that a male was resighted. For analyses partitioned into
192 territorial and nonterritorial episodes, the exposure term was the number of days males held or
193 did not hold territories during the study.

194 To include repeated measurements on individuals when available, we used mixed-effect
195 models with random intercepts for individual IDs. We used R [35] to conduct all statistical
196 analyses, using the packages MASS [36] for negative binomial regression, survival [37,38] for
197 survival analyses, glmmADMB [39,40] and lme4 [41] for mixed-effect GLMs, pscl for zero-
198 inflation models [42,43], and ggplot2 [44] for figures.

199

200 **3. Results**

201

202 (a) *Sample sizes and preliminary results*

203 We marked and included 146 *H. americana* males in the experiment, recorded 444
204 unique *H. americana* mating events involving marked males (82 failed mating attempts; 362
205 successful matings; mean number of successful matings per male = 3.26, s.d. = 4.74), and made
206 1207 observations of fights involving at least one *H. americana* male. We resighted 111 males,
207 or 76.03% of the number marked, at least one day after marking, and resighted males' locations
208 were recorded an average of 12.1 times per day. Among these resighted males, the median
209 number of days resighted was 6, and most were resighted every day prior to their final
210 disappearance (mean proportion of days on which males were resighted = 0.93). We witnessed 5
211 failed mating attempts of *H. americana* males with *H. titia* females; a tandem was successfully
212 formed in 3 of these cases but broke prior to copulation. In no cases did the sham (clear) and
213 unmanipulated control groups differ significantly from each other (see electronic supplementary
214 material), and thus the control groups were pooled for comparison to the experimental
215 (blackened) group.

216

217 *(b) Female mate recognition*

218 There was no overall effect of the wing blackening treatment on: (1) the proportion of
219 attempted tandems that resulted in a successful mating (Fig. 3a, mixed effect binomial model of
220 tandems [success = 1, failure = 0] with a random intercept for male IDs, treatment $n = 444$, $z = -$
221 0.14 , $p = 0.89$), (2) the rate of successful matings (Fig. 3b, negative binomial model of the count
222 of matings with an offset term for the log of the total number of days resighted, treatment d.f. =
223 110 , $z = -1.02$, $p = 0.31$), or (3) the duration of copulatory wheels (Fig. 3c, mixed effect model of
224 the logarithm of the duration of copulatory wheels with a random intercept for male IDs,
225 treatment $n = 119$, $z = 0.26$, $p = 0.8$).

226 In *H. americana*, holding a territory is not essential for mating but males generally mate
227 more often when they hold a territory [27]. Thus, a male's mating rate is influenced by the
228 proportion of his life spent holding a territory. In this experiment, males mated 2.1 times more
229 frequently when they held a territory than when they did not (zero-inflated negative binomial
230 model of the count of successful matings with a random intercept for male ID, $n = 180$, $z = 5.03$,
231 $p < 0.001$). To separate effects of territory competition from female choice, we partitioned
232 males' careers into territorial and nonterritorial episodes to further examine the effect of the
233 experimental treatment on male mating success [27]. In other words, differences in mating
234 success between treatments could be a result of males of one treatment being unable to hold
235 territories, a phenomenon independent of female mate recognition. When males held territories,
236 neither the proportion of attempted tandems that resulted in a successful mating (mixed effect
237 binomial model of tandems [success = 1, failure = 0] with a random intercept for male IDs, $n =$
238 353 , treatment $z = -0.50$, $p = 0.62$) nor the mating rate (negative binomial model of the count of
239 matings with an offset term for the log of the total number of days territorial, treatment d.f. = 71,
240 $z = -1.69$, $p = 0.092$) were influenced by the experimental treatment. Likewise, when males did
241 not hold territories, the proportion of successful tandems did not depend on treatment (mixed
242 effect binomial model of tandems [success = 1, failure = 0] with a random intercept for male IDs,
243 $n = 91$, treatment $z = 0.89$, $p = 0.37$). However, the mating rate of non-territory holding
244 blackened males was 1.74 times higher than that of controls (negative binomial model of the
245 count of matings with an offset term for the log of the total number of days non-territorial,
246 treatment d.f. = 107, $z = -1.992$, $p = 0.046$).

247 The post-copulatory behaviour of females did not distinguish among males based on their
248 treatments. Neither a female's first nor last mate of the day depended on the male's treatment

249 group (estimated from intercept of a mixed-effect model of first or last male treatment with a
250 random intercept for female ID, both $p > 0.05$, see electronic supplementary material). Similarly,
251 the treatment of a female's mates did not influence her probability of remating within one day
252 (Fig. 3d, blackened versus control in a binomial mixed-effect model of remating with a random
253 intercept for female ID, $n = 255$, $z = 0.82$, $p = 0.41$) or within three days (Fig. 3d, blackened
254 versus control in a binomial mixed-effect model of remating with a random intercept for female
255 ID, $n = 255$, $z = 1.28$, $p = 0.20$). Furthermore, the treatment of the male with which a female
256 remated was not influenced by the treatment of her previous mate, whether analyzed within one
257 day (binomial lag model with a lag variable for the subsequent mate treatment used as a predictor
258 with a random intercept for female/1day, $n = 76$, $z = -0.811$, $p = 0.42$) or over a three day period
259 (binomial lag model with a lag variable for the subsequent mate treatment used as a predictor
260 with a random intercept for female/3day, $n = 141$, $z = -0.784$, $p = 0.43$).

261

262 (c) Treatment effects on fighting, disappearance, and territory tenure

263 Compared to controls, blackened *H. americana* males were more likely to fight with *H.*
264 *titia* males, with an increasing effect of treatment in escalated fights and for males who were
265 resighted within 4 m of *H. titia* territory holders (Table 1). We found little evidence for an effect
266 of the experimental treatment on intraspecific fighting rates (Table 1). In the analysis for which
267 we reduced all fights between the same two males to a single observation, there was a marginally
268 significant trend for blackened males to be involved in more intraspecific fights than control
269 males, but this effect disappeared when the analysis was restricted to escalated fights, and there
270 was no such trend in the other two datasets (Table 1, electronic supplementary material, Fig. S2).

271 The rate at which blackened males disappeared from the study transects was 1.57 times
272 higher than that of controls (Cox proportional hazard treatment coefficient = 0.4541, $n = 146$, $z =$
273 2.549, $p = 0.011$; limiting analysis to clear and blackened treatments, Cox proportional hazard
274 treatment coefficient = 0.474, $n = 110$, $z = 2.12$, $p = 0.034$). Among all males that were resighted
275 at least once, however, there was no difference in the disappearance rate of blackened males and
276 controls (Cox proportional hazard treatment coefficient = 0.3531, $n = 111$, $z = 1.694$, $p = 0.09$;
277 limiting analysis to clear and black ink treatments, Cox proportional hazard treatment coefficient
278 = 0.432, $n = 80$, $z = 1.65$, $p = 0.10$).

279 Experimentally blackened males were just as likely as control males to perch and defend
280 territories near heterospecifics (see electronic supplementary material). However, blackened
281 males suffered a survival cost from interacting with *H. titia* males; blackened males whose
282 median perch locations were ever within 4 m of *H. titia* males had 1.9 times higher
283 disappearance rates than control males (Fig. 2, Cox proportional hazard treatment coefficient =
284 0.643, $n = 62$, $z = 2.154$, $p = 0.031$; limiting analysis to clear and black ink treatments, Cox
285 proportional hazard treatment coefficient = 0.992, $n = 42$, $z = 2.37$, $p = 0.018$). Experimentally
286 blackened males also held territories for fewer days than control males when they were ever
287 within 4 m of *H. titia* males, but experienced no such difference when they were never within 4
288 m of *H. titia* males (negative binomial model of territorial days with offset term for the log
289 number of total days resighted, treatment*proximity d.f. = 110, $z = -2.427$, $p = 0.015$).

290

291 **4. Discussion**

292 Female mate recognition appeared to be unaffected by the species difference in male
293 wing coloration. Manipulating *H. americana* male wings to appear similar to those of *H. titia*

294 males had no discernable effect on mating—females neither rejected experimentally blackened
295 males more often after being clasped nor mated less often with experimentally blackened males
296 (Fig. 2a,b). The only hint of an effect of the experimental treatment on female responses was
297 opposite to the predicted direction: among non-territory holders, blackened males mated at a
298 marginally significantly higher rate than controls. Mating rates in the present study are similar to
299 those measured previously in *H. americana* [33].

300 Post-copulatory means of discrimination are possible in calopterygid damselflies, where
301 there is extensive evidence that males remove stored sperm from females during copulation [46–
302 48]. If the amount of time spent in copula is under female control (but see [49–51]), females may
303 be able to control how much sperm from previous mates is removed by her current mate, the
304 amount of sperm that the male is able to transfer, or the amount of time she spends with the
305 current male at the expense of time for future matings [45,52,53]. Yet, in our study, copulation
306 duration was also unaffected by the experimental treatment. Since females are sometimes
307 clasped by different males after emerging from ovipositing, cryptic female choice may take the
308 form of females biasing either first or last matings toward particular males, remating more often
309 after mating with a non-preferred male [45,54], or similarly, biasing remating toward a particular
310 treatment, yet none of these indicators of cryptic female choice occurred in our experiment,
311 whether we analyzed single days or three day windows for each female (given the possibility of
312 sperm storage across days of a female’s reproductive bout). We did not test the possibility that
313 females discriminated between the treatments via some other cryptic choice mechanism such as
314 biasing paternity sperm storage [45,54,55] or manipulating fecundity [56,57].

315 *H. americana* females may use traits other than wing coloration to differentiate between
316 conspecific and heterospecific males. In *Enallagma* damselflies, the appendages that males use

317 to clasp females (cerci) have evolved in a correlated fashion with the corresponding structures on
318 females—consistent with the hypothesis that these structures are involved in species recognition
319 [58,59]. Female *Hetaerina* may also use tactile information from male cerci and/or paraprocts
320 (i.e., inferior and superior clasping appendages), as these structures are highly variable and
321 species specific [32].

322 In agreement with previous research [25], manipulating the wings of *H. americana* males
323 to resemble those of *H. titia* males increased the occurrence of interspecific fighting. We further
324 documented effects of the experimental manipulation on the rate and intensity of interspecific
325 fights and the proportion of a male's life during which he held a territory. Moreover, blackened
326 *H. americana* males in our study close in proximity to *H. titia* males suffered reduced survival
327 compared to control males, likely resulting from the increase in fights with heterospecific males.
328 We also documented an immediate effect of the phenotype manipulation: a reduction in the
329 probability that blackened males were resighted. Whether this early attrition of blackened males
330 reflects mortality or dispersal is not clear, but if weaker/lower quality males were more likely to
331 be lost from the study, this might account for the relatively high non-territorial mating rates of
332 the remaining blackened males.

333 Together, our results strengthen the hypothesis that previously documented shifts in both
334 competitor recognition and male wing coloration [24–26] have resulted from ACD. One previous
335 study documented a pattern of character displacement in male breeding coloration of benthic and
336 limnetic forms of three-spined sticklebacks (*Gasterosteus aculeatus*) [60] that cannot be
337 explained by a shift in female preferences or colour sensitivity [61,62], effectively ruling out
338 RCD as a potential explanation for the observed shift. Nevertheless, to our knowledge, the
339 current study is the first to experimentally demonstrate that a target of male competitor

340 recognition is not also a target of female mate recognition and thus the first to support ACD over
341 RCD as the cause of a character displacement pattern. Although some investigators have grouped
342 character displacement influencing interspecific aggression under RCD (e.g. [2]), this study
343 highlights the mechanistic distinction between RCD and ACD: our phenotype manipulation
344 experiment confirmed that the species difference *H. americana* male wing coloration influences
345 interspecific aggression but does not influence female mate recognition. Based on these results,
346 we can reject the hypothesis that previously documented sympatric shifts in male traits are
347 byproducts of RCD.

348

349 **Acknowledgements.** We thank Simone Giovanetti, Sara Hu, and Linnea Karlen for field
350 assistance, Cynthia Gonzalez for help with data processing, and Andy Lin at UCLA Stats
351 Consulting for statistical help. Adrea Gonzales-Karlsson, Kathryn Peiman, Thomas B. Smith,
352 Erik Svensson, and an anonymous reviewer provided helpful feedback on the manuscript. J.P.D.
353 received an NSF Graduate Research Fellowship and fellowship support from the UCLA
354 Graduate Division and Department of Ecology & Evolutionary Biology. This research was
355 funded by NSF DEB-1020586 to G.F.G.

356

357 **Data Accessibility.** Data sets used in our analyses are available at Dryad (dryad.org):

358 doi:10.5061/dryad.158pj

359

360 **References**

- 361 1 Brown, W. L. & Wilson, E. O. 1956 Character displacement. *Syst. Zool.* **5**, 49–64.
- 362 2 Pfennig, K. S. & Pfennig, D. W. 2009 Character displacement: ecological and
363 reproductive responses to a common evolutionary problem. *Q. Rev. Biol.* **84**, 253–276.
- 364 3 Wallace, A. R. 1889 *Darwinism*. 2007 facsi. New York: Cosimo, Inc.
- 365 4 Dobzhansky, T. 1937 *Genetics and the origin of species*. 1982 Repr. New York:
366 Columbia University Press.
- 367 5 Dobzhansky, T. 1940 Speciation as a stage in evolutionary divergence. *Am. Nat.* **74**, 312–
368 321. (doi:10.1086/282871)
- 369 6 Coyne, J. & Orr, H. 2004 *Speciation*. Sunderland, MA: Sinauer Associates, Inc.
- 370 7 Gröning, J. & Hochkirch, A. 2008 Reproductive interference between animal species. *Q.*
371 *Rev. Biol.* **83**, 257–82.
- 372 8 Peiman, K. & Robinson, B. 2010 Ecology and evolution of resource-related heterospecific
373 aggression. *Q. Rev. Biol.* **85**, 133–158.
- 374 9 Grether, G. F., Losin, N., Anderson, C. N. & Okamoto, K. 2009 The role of interspecific
375 interference competition in character displacement and the evolution of competitor
376 recognition. *Biol. Rev.* **84**, 617–635. (doi:10.1111/j.1469-185X.2009.00089.x)
- 377 10 Grether, G., Anderson, C., Drury, J. P., Losin, N., Peiman, K. & Okamoto, K. 2013 The
378 evolutionary consequences of interspecific aggression. *Ann. NY Acad. Sci.* **1289**, 48–68.
- 379 11 Berglund, A., Bisazza, A. & Pilastro, A. 1996 Armaments and ornaments: an evolutionary
380 explanation of traits of dual utility. *Biol. J. Linn. Soc.* **58**, 385–399. (doi:10.1111/j.1095-
381 8312.1996.tb01442.x)

- 382 12 Okamoto, K. & Grether, G. F. 2013 The evolution of species recognition in competitive
383 and mating contexts: The relative efficacy of alternative mechanisms of character
384 displacement. *Ecol. Lett.* **16**, 670–678.
- 385 13 Calhim, S., Adamik, P., Järvisistö, P., Leskinen, P., Török, J., Wakamatsu, K. & Laaksonen,
386 T. 2014 Heterospecific female mimicry in *Ficedula* flycatchers. *J. Evol. Biol.* **27**, 660–
387 666. (doi:10.1111/jeb.12328)
- 388 14 Král, M., Järvi, T. & Bicik, V. 1988 Inter-specific aggression between the collared
389 flycatcher and the pied flycatcher: The selective agent for the evolution of light-coloured
390 male pied flycatcher populations? *Ornis Scand.* **19**, 287–289.
- 391 15 Sætre, G.-P., Král, M. & Bicik, V. 1993 Experimental evidence for interspecific female
392 mimicry in sympatric *Ficedula* flycatchers. *Evolution (N. Y.)*. **47**, 939–945.
- 393 16 Alatalo, R. V., Gustafsson, L. & Lundberg, A. 1994 Male coloration and species
394 recognition in sympatric flycatchers. *Proc. R. Soc. B Biol. Sci.* **256**, 113–118.
395 (doi:10.1098/rspb.1994.0057)
- 396 17 Sætre, G.-P., Moum, T., Stanislav, B., Král, M., Adamjan, M. & Moreno, J. 1997 A
397 sexually selected character displacement in flycatchers reinforces premating isolation.
398 **387**, 1995–1998.
- 399 18 Tynkkynen, K., Rantala, M. J. & Suhonen, J. 2004 Interspecific aggression and character
400 displacement in the damselfly *Calopteryx splendens*. *J. Evol. Biol.* **17**, 759–67.
401 (doi:10.1111/j.1420-9101.2004.00733.x)
- 402 19 Honkavaara, J., Dunn, D. W., Ilvonen, S. & Suhonen, J. 2010 Sympatric shift in a male
403 sexual ornament in the damselfly *Calopteryx splendens*. *J. Evol. Biol.* **24**, 139–45.
404 (doi:10.1111/j.1420-9101.2010.02146.x)

- 405 20 Tynkkynen, K., Kotiaho, J. S., Luojumäki, M. & Suhonen, J. 2006 Interspecific
406 territoriality in *Calopteryx* damselflies: the role of secondary sexual characters. *Anim.*
407 *Behav.* **71**, 299–306. (doi:10.1016/j.anbehav.2005.03.042)
- 408 21 Tynkkynen, K., Kotiaho, J. S., Luojumäki, M. & Suhonen, J. 2005 Interspecific
409 aggression causes negative selection on sexual characters. *Evolution (N. Y.)*. **59**, 1838–
410 1843.
- 411 22 Svensson, E. I., Karlsson, K., Friberg, M. & Eroukhmanoff, F. 2007 Gender differences in
412 species recognition and the evolution of asymmetric sexual isolation. *Curr. Biol.* **17**,
413 1943–7. (doi:10.1016/j.cub.2007.09.038)
- 414 23 Svensson, E. I., Eroukhmanoff, F., Karlsson, K., Runemark, A. & Brodin, A. 2010 A role
415 for learning in population divergence of mate preferences. *Evolution (N. Y.)*. **64**, 3101–13.
416 (doi:10.1111/j.1558-5646.2010.01085.x)
- 417 24 Anderson, C. N. & Grether, G. F. 2010 Interspecific aggression and character
418 displacement of competitor recognition in *Hetaerina* damselflies. *Proc. R. Soc. B Biol.*
419 *Sci.* **277**, 549–55. (doi:10.1098/rspb.2009.1371)
- 420 25 Anderson, C. N. & Grether, G. F. 2011 Multiple routes to reduced interspecific territorial
421 fighting in *Hetaerina* damselflies. *Behav. Ecol.* **22**, 527–534. (doi:10.1093/beheco/arr013)
- 422 26 Anderson, C. N. & Grether, G. F. 2010 Character displacement in the fighting colours of
423 *Hetaerina* damselflies. *Proc. R. Soc. B Biol. Sci.* **277**, 3669–75.
424 (doi:10.1098/rspb.2010.0935)
- 425 27 Grether, G. F. 1996 Intrasexual competition alone favors a sexually dimorphic ornament
426 in the rubyspot damselfly *Hetaerina americana*. *Evolution (N. Y.)*. **50**, 1949–1957.

- 427 28 Córdoba-Aguilar, A., Raihani, G., Serrano-Meneses, A. & Contreras-Garduño, J. 2009
428 The lek mating system of *Hetaerina* damselflies (Insecta: Calopterygidae). *Behaviour* **146**,
429 189–207. (doi:10.1163/156853909X410739)
- 430 29 Anderson, C. N., Córdoba-Aguilar, A., Drury, J. P. & Grether, G. F. 2011 An assessment
431 of marking techniques for odonates in the family Calopterygidae. *Entomol. Exp. Appl.*
432 **141**, 258–261. (doi:10.1111/j.1570-7458.2011.01185.x)
- 433 30 Kemp, D. J., Jones, D., Macedonia, J. M. & Krockenberger, A. K. 2014 Female mating
434 preferences and male signal variation in iridescent *Hypolimnas* butterflies. *Anim. Behav.*
435 **87**, 221–229. (doi:10.1016/j.anbehav.2013.11.001)
- 436 31 Grether, G. F. 1995 Natural and sexual selection on wing coloration in the rubyspot
437 damselfly *Hetaerina americana*. Ph.D. Dissertation, University of California, Davis.
- 438 32 Garrison, R. W. 1990 A synopsis of the genus *Hetaerina* with descriptions of four new
439 species. *Trans. Am. Entomol. Soc.* **116**, 175–259.
- 440 33 Grether, G. 1996 Sexual selection and survival selection on wing coloration and body size
441 in the rubyspot damselfly *Hetaerina americana*. *Evolution (N. Y.)*. **50**, 1939–1948.
- 442 34 Johnson, C. 1963 Interspecific territoriality in *Hetaerina americana* (Fabricius) and *H.*
443 *titia* (Drury)(Odonata: Calopterygidae) with a preliminary analysis of the wing color
444 pattern variation. *Can. Entomol.* **95**, 575–582.
- 445 35 R Core Team 2013 R: A Language and Environment for Statistical Computing.
- 446 36 Venables, W. N. & Ripley, B. D. 2002 *Modern Applied Statistics with S*. Fourth. New
447 York: Springer.
- 448 37 Therneau, T. & Grambsch, P. 2000 *Modeling Survival Data: Extending the Cox Model*.
449 New York: Springer.

450 38 Therneau, T. 2013 A Package for Survival Analysis in S (R package version 2.37-4).

451 39 Fournier, D., Skaug, H., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., Nielsen, A.
452 & Sibert, J. 2012 AD model builder: Using automatic differentiation for statistical
453 inference of highly parameterized complex nonlinear models. *Optim. Methods Softw.* **27**,
454 233–249.

455 40 Skaug, H., Fournier, D., Nielsen, A., Magnusson, A. & Bolker, B. 2012 Generalized linear
456 mixed models using AD model builder (R package version 0.7.2.12).

457 41 Bates, D., Maechler, M. & Bolker, B. 2012 lme4: Linear mixed-effects models using S4
458 classes.

459 42 Jackman, S. 2012 pscl: Classes and Methods for R Developed in the Political Science
460 Computational Laboratory, Stanford University.

461 43 Zeileis, A., Kleiber, C. & Jackman, S. 2008 Regression models for count data in R. *J. Stat.*
462 *Softw.* **27**.

463 44 Wickham, H. 2009 *ggplot2: elegant graphics for data analysis*. Springer New York.

464 45 Eberhard, W. G. 1996 *Female control: Sexual selection by cryptic female choice*.
465 Princeton University Press.

466 46 Waage, J. K. 1979 Dual function of the damselfly penis: Sperm removal and transfer.
467 *Science (80-.)*. **203**, 916.

468 47 Siva-Jothy, M. T. & Hooper, R. E. 1995 The disposition and genetic diversity of stored
469 sperm in females of the damselfly *Calopteryx splendens xanthostoma* (Charpentier). *Proc.*
470 *R. Soc. B Biol. Sci.* **259**, 313–318. (doi:10.1098/rspb.1995.0046)

- 471 48 Córdoba-Aguilar, A. 1999 Male copulatory sensory stimulation induces female ejection of
472 rival sperm in a damselfly. *Proc. R. Soc. B Biol. Sci.* **266**, 779–784.
473 (doi:10.1098/rspb.1999.0705)
- 474 49 Miller, P. 1987 An examination of the prolonged copulations of *Ischnura elegans* (Vander
475 Linden) (Zygoptera: Coenagrionidae). *Odonatologica* **16**, 37–56.
- 476 50 Siva-Jothy, M. & Tsubaki, Y. 1989 Variation in copulation duration in *Mnais pruinosa*
477 *pruinosa* Selys (Odonata: Calopterygidae): 2. Causal factors. *Behav. Ecol. Sociobiol.* **25**,
478 261–267.
- 479 51 Fincke, O. M. 1997 Conflict resolution in the Odonata: Implications for understanding
480 female mating patterns and female choice. *Biol. J. Linn. Soc.* **60**, 201–220.
- 481 52 Michiels, N. K. 1992 Consequences and adaptive significance of variation in copulation
482 duration in the dragonfly *Sympetrum danae*. *Behav. Ecol. Sociobiol.* **29**, 429–435.
- 483 53 Andrés, J. & Cordero-Rivera, A. 2000 Copulation duration and fertilization success in a
484 damselfly: an example of cryptic female choice? *Anim. Behav.* **59**, 695–703.
485 (doi:10.1006/anbe.1999.1372)
- 486 54 Eberhard, W. G. 2000 Criteria for demonstrating postcopulatory female choice. **54**, 1047–
487 1050.
- 488 55 Siva-Jothy, M. & Hooper, R. 1996 Differential use of stored sperm during oviposition in
489 the damselfly *Calopteryx splendens xanthostoma* (Charpentier). *Behav. Ecol. Sociobiol.*
490 **39**, 389–393.
- 491 56 Burley, N. 1988 The differential-allocation hypothesis: An experimental test. *Am. Nat.*
492 **132**, 611–628.

- 493 57 Gowaty, P. A., Anderson, W. W., Bluhm, C. K., Drickamer, L. C., Kim, Y.-K. & Moore,
494 A. J. 2007 The hypothesis of reproductive compensation and its assumptions about mate
495 preferences and offspring viability. *Proc. Natl. Acad. Sci.* **104**, 15023–15027.
496 (doi:10.1073/pnas.0706622104)
- 497 58 McPeck, M. A., Shen, L., Torrey, J. Z. & Farid, H. 2008 The tempo and mode of three-
498 dimensional morphological evolution in male reproductive structures. *Am. Nat.* **171**,
499 E158–E178. (doi:10.1086/587076)
- 500 59 McPeck, M. A., Shen, L. & Farid, H. 2009 The correlated evolution of three-dimensional
501 reproductive structures between male and female damselflies. *Evolution (N. Y.)*. **63**, 73–83.
502 (doi:10.1111/j.1558-5646.2008.00527.x)
- 503 60 Albert, A. Y. K., Millar, N. P. & Schluter, D. 2007 Character displacement of male nuptial
504 colour in threespine sticklebacks (*Gasterosteus aculeatus*). *Biol. J. Linn. Soc.* **91**, 37–48.
505 (doi:10.1111/j.1095-8312.2007.00777.x)
- 506 61 Boughman, J. W. 2001 Divergent sexual selection enhances reproductive isolation in
507 sticklebacks. *Nature* **411**, 944–948.
- 508 62 Boughman, J., Rundle, H. & Schluter, D. 2005 Parallel evolution of sexual isolation in
509 sticklebacks. *Evolution (N. Y.)*. **59**, 361–373.

510

511 **Table & Figure Legends**

512

513 **Table 1.** Effects of the experimental treatment on intraspecific and interspecific fighting rates.

514

515 **Figure 1.** Photographs of a representative (a) unmanipulated *Hetaerina americana* male, (b) a *H.*
516 *titia* male, and *H. americana* males with (c) clear ink and (d) black ink on their hindwings. All
517 males shown here were photographed during the course of the experiment.

518

519 **Figure 2.** Effects of the experimental treatment on survival of males seen in close proximity to
520 *H. titia* males. Kaplan-Meier plot, crosshatches indicate censored data points.

521

522 **Figure 3.** Lack of an effect of the experimental treatment on overall male mating success,
523 measured either as (a) the proportion of successful tandems, (b) the mating rates of males, (c) the
524 length of the copulatory wheel, or (d) the probability of a female remating within one or three
525 days. In panels (a)-(c), black dots indicate blackened males, grey dots indicate males with clear
526 ink, empty circles indicate unmanipulated males, and horizontal lines represent group means. In
527 panel (d), black bars represent blackened males and empty bars represent control males.

528

529

530 **Table 1.** Effects of the experimental treatment on intraspecific and interspecific fighting rates.

531

data set	intraspecific fights (<i>H. americana</i> v. <i>H. americana</i>)		interspecific fights (<i>H. americana</i> v. <i>H. titia</i>)	
	all fight types	only escalated fights	all fight types	only escalated fights
1) one fight per dyad ¹	<i>n</i> = 666, $\chi^2 = 4.17$, <i>p</i> = 0.041	<i>n</i> = 374, $\chi^2 = 0.22$, <i>p</i> = 0.64	<i>n</i> = 115, $\chi^2 = 4.69$, <i>p</i> = 0.00099	<i>n</i> = 82, $\chi^2 = 11.66$, <i>p</i> = 0.00064
2) one fight per dyad per day ²	d.f. = 81, <i>z</i> = 1.07, <i>p</i> = 0.28	d.f. = 81, <i>z</i> = 0.18, <i>p</i> = 0.86	d.f. = 81, <i>z</i> = 2.38, <i>p</i> = 0.017, (1.94)	d.f. = 81, <i>z</i> = 2.92, <i>p</i> = 0.0035, (2.5)
3) all fight observations ²	d.f. = 81, <i>z</i> = 0.18, <i>p</i> = 0.86	d.f. = 81, <i>z</i> = -0.48, <i>p</i> = 0.63	d.f. = 55, <i>z</i> = 3.01, <i>p</i> = 0.0026, (2.25)	d.f. = 55, <i>z</i> = 3.53, <i>p</i> = 0.00043, (2.96)
			d.f. = 81, <i>z</i> = 2.82, <i>p</i> = 0.0049, (2.36)	d.f. = 81, <i>z</i> = 3.02, <i>p</i> = 0.0026, (2.76)
			d.f. = 55, <i>z</i> = 3.38, <i>p</i> = 0.00072, (2.75)	d.f. = 55, <i>z</i> = 3.52, <i>p</i> = 0.00043, (3.26)

532 (Statistical tests compare blackened and control males. Data sets correspond to those described in

533 the main text. Analyses presented in italics restrict males to those seen within 4 m of a territorial

534 *H. titia* male. The ratios of blackened male interspecific fighting rates to control male

535 interspecific fighting rates are presented in parentheses.)

536 ¹Chi-squared goodness-of-fit test of count of fights, comparing experimental versus control to a

537 null expectation of fights based on the resighting record (see electronic supplementary material)

538 ²Negative binomial model of the number of interspecific fights, offset by the log of the number

539 of days territorial or fighting (if larger)

540