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

22 Traits that mediate intraspecific social interactions may overlap in closely related sympatric species, resulting in costly between-species interactions. Such interactions have 23 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**

When closely related species come into secondary contact, they may overlap in traits
used as intraspecific social signals, resulting in costly interactions between species. Evolutionary
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 between species, which in animals usually takes the form of aggressive interactions, is also very 46 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

to *C. splendens* males with relatively larger wing spots in sympatry, which consequently affects
male fitness [18,20,21], yet female mate recognition is also influenced by male wing coloration
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 though 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.

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

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

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

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

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

137

138 (c) Behavioural observations

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

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

# 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 resigning 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 resigned 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 N180 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 ( $\leq 4$  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  $\leq 2$  m and that adjacent territories are typically  $\leq 2$  m apart, as reported previously 188 [25].

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

To include repeated measurements on individuals when available, we used mixed-effect models with random intercepts for individual IDs. We used R [35] to conduct all statistical analyses, using the packages MASS [36] for negative binomial regression, survival [37,38] for survival analyses, glmmADMB [39,40] and lme4 [41] for mixed-effect GLMs, pscl for zeroinflation 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 resignted males' locations 208 were recorded an average of 12.1 times per day. Among these resignted males, the median 209 number of days resignted was 6, and most were resignted every day prior to their final 210 disappearance (mean proportion of days on which males were resigned = 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 resigned, 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, p < 0.001). To separate effects of territory competition from female choice, we partitioned 231 232 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 =353, treatment z = -0.50, p = 0.62) nor the mating rate (negative binomial model of the count of 238 239 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).

The post-copulatory behaviour of females did not distinguish among males based on theirtreatments. 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 resignted 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 higher than that of controls (Cox proportional hazard treatment coefficient = 0.4541, n = 146, z =272 2.549, p = 0.011; limiting analysis to clear and blackened treatments, Cox proportional hazard 273 274 treatment coefficient = 0.474, n = 110, z = 2.12, p = 0.034). Among all males that were resignted 275 at least once, however, there was no difference in the disappearance rate of blackened males and controls (Cox proportional hazard treatment coefficient = 0.3531, n = 111, z = 1.694, p = 0.09; 276 277 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 proportional hazard treatment coefficient = 0.992, n = 42, z = 2.37, p = 0.018). Experimentally 285 286 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 number of total days resignted, treatment\*proximity d.f. = 110, z = -2.427, p = 0.015). 289

290

### **4. Discussion**

Female mate recognition appeared to be unaffected by the species difference in male wing coloration. Manipulating *H. americana* male wings to appear similar to those of *H. titia*  males had no discernable effect on mating—females neither rejected experimentally blackened
males more often after being clasped nor mated less often with experimentally blackened males
(Fig. 2a,b). The only hint of an effect of the experimental treatment on female responses was
opposite to the predicted direction: among non-territory holders, blackened males mated at a
marginally significantly higher rate than controls. Mating rates in the present study are similar to
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].

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

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

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

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357 Data Accessibility. Data sets used in our analyses are available at Dryad (dryad.org):
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511	Table	&	Figure	Legends

513	<b>Table 1.</b> Effects of the experimental treatment on intraspecific and interspecific fighting rates.
514	
515	Figure 1. Photographs of a representative (a) unmanipulated <i>Hetaerina americana</i> male, (b) a <i>H</i> .
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	

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

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

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.)

<sup>1</sup>Chi-squared goodness-of-fit test of count of fights, comparing experimental versus control to a

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

<sup>2</sup>Negative binomial model of the number of interspecific fights, offset by the log of the number

539 of days territorial or fighting (if larger)