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1 **TITLE:** Mechanisms of reduced interspecific interference between territorial species

2

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16

17 **ABSTRACT:** Interspecific territoriality has complex ecological and evolutionary

18 consequences. Species that interact aggressively often exhibit spatial or temporal shifts in

19 activity that reduce the frequency of costly encounters. We analyzed data collected over a

20 13-year period on 50 populations of rubyspot damselflies (*Hetaerina* spp.) to examine

21 how rates of interspecific fighting covary with fine-scale habitat partitioning and to test

22 for agonistic character displacement in microhabitat preferences. In most sympatric

23 species, interspecific fights occur less frequently than expected based on the species'
24 relative densities. Incorporating measurements of spatial segregation and species
25 discrimination into the calculation of expected frequencies accounted for most of the
26 reduction in interspecific fighting (subtle differences in microhabitat preferences could
27 account for the rest). In 23 of 25 sympatric population pairs, we found multivariate
28 differences between species in territory microhabitat (perch height, stream width, current
29 speed, and canopy cover). As predicted by the agonistic character displacement
30 hypothesis, sympatric species that respond more aggressively to each other in direct
31 encounters differ more in microhabitat use and have higher levels of spatial segregation.
32 Previous work established that species with the lowest levels of interspecific fighting
33 have diverged in territory signals and competitor recognition through agonistic character
34 displacement. In the other species pairs, interspecific aggression appears to be maintained
35 as an adaptive response to reproductive interference, but interspecific fighting is still
36 costly. We now have robust evidence that evolved shifts in microhabitat preferences also
37 reduce the frequency of interspecific fighting.

38

39 **KEYWORDS:** agonistic character displacement, habitat partitioning, habitat preference,
40 microhabitat, interference competition, interspecific territoriality, Odonata

41

42 **INTRODUCTION**

43 Competition between animal taxa is widespread and often involves aggression.
44 Interspecific aggression may initially arise from misdirected intraspecific aggression
45 (Murray 1971; Nishikawa 1987; Singer 1989; Schultz and Switzer 2001; Korner et al.
46 2010; Cowen et al. 2020). However, in the absence of a contested resource, species are
47 expected to diverge in ways that reduce the frequency and costs of interspecific
48 aggression, a process known as divergent agonistic character displacement (ACD). Most
49 documented cases of divergent ACD involve evolutionary shifts in agonistic signals and
50 competitor recognition (Grether et al. 2009; Grether et al. 2013; Grether et al. 2017;
51 Latour and Ganem 2017; Moran and Fuller 2018a; Moran and Fuller 2018b; Zambre et
52 al. 2020). Conversely, species with a contested resource in common may converge in
53 agonistic signals and competitor recognition to facilitate resource defense and
54 partitioning, i.e., convergent ACD (Cody 1973; Grether et al. 2009; Reif et al. 2015;
55 Pasch et al. 2017; Souriau et al. 2018; Kirschel et al. 2019). Interspecific mate
56 competition arising from reproductive interference has also been shown to cause
57 convergent ACD (Drury, Okamoto, et al. 2015; Grether et al. 2020).

58 In addition to convergence or divergence in traits related to competitor
59 recognition, interspecific aggression can also cause drastic changes in species' spatial and
60 temporal niches (Melville 2002; Gotelli et al. 2010; Pigot and Tobias 2013; Edgehouse et
61 al. 2014; Ulrich et al. 2017; Eurich et al. 2018). One common result of interspecific
62 territoriality is competitive displacement where a dominant or more aggressive species
63 forces a subordinate species into a different habitat or to be active during different

64 periods (Garcia 1983, Reitz and Trumble 2002, Langkilde and Shine 2004, Jankowski et
65 al. 2010, Pasch et al. 2013, Kajtoch et al. 2015). In other cases, both species may shift in
66 habitat use or temporal activity (Bay et al. 2001; Eurich et al. 2018; Reif et al. 2018).
67 Whether the shift in activity occurs in one or both species, interspecific competition is
68 reduced, although one species may be forced into suboptimal habitat (Randall 1978;
69 Robinson and Terborgh 1995; Grether et al. 2013). Habitat partitioning can occur at
70 various spatial and temporal scales, such as elevational or latitudinal gradients on a
71 macroscale (Connell 1983; Schoener 1983; Lewin 1989; Hawkins 1999; Mark et al.
72 2001) as well as across small scale variation in microhabitat (Eurich et al. 2018; Reif et
73 al. 2018).

74 Habitat partitioning could also arise from species differences in habitat
75 preferences that evolved in response to selection against interspecific fighting in the past,
76 which would be a form of divergent ACD (Grether et al. 2009). As yet, however, there
77 are few if any well documented cases of ACD in habitat preferences (for a possible
78 example, see Vallin et al. 2012). Species can diverge in habitat use for many reasons, and
79 determining whether these differences are products of past or ongoing interspecific
80 interactions is challenging (Connell 1978; Ross 1986; Wisheu 1998; Pinter-Wollman et
81 al. 2006).

82 Rubyspot damselflies (*Hetaerina*) are a good system for examining the
83 relationship between interspecific aggression and niche partitioning because levels of
84 interspecific aggression vary widely among sympatric species. Male rubyspots defend
85 mating territories along streams and rivers (Johnson 1963; Córdoba-Aguilar et al. 2009;

86 Anderson and Grether 2011; but see Guillermo-Ferreira and Del-Claro 2011). Mature
87 males of all species in the genus have red pigmentation at the base of their wings,
88 whereas the lack of red or other conspicuous pigmentation in female wings makes them
89 more cryptic (Garrison 1990). Simulated territory intrusion and wing color manipulation
90 experiments have shown that the responses of territory holders to intruders is largely
91 based on wing coloration and that species with more similar wing coloration respond
92 more aggressively to each other (Anderson and Grether 2010a; Drury and Grether 2014;
93 Grether et al. 2015).

94 Interspecific aggression in *Hetaerina* appears to largely be an adaptive response
95 to interspecific mate competition (Drury, Okamoto, et al. 2015; Drury, Anderson, et al.
96 2019; Grether et al. 2020), but selection should still favor adaptations that reduce the
97 frequency of interspecific fighting. Territorial fights are costly, primarily because they
98 can result in males losing their territories and priority of access to ovipositing females
99 (territory possession confers a three-fold mating advantage; Grether 1996; Drury &
100 Grether 2014). Damselfly fights also have energetic and physiological costs (reviewed in
101 Suhonen et al. 2008; Vieira and Peixoto 2013; Córdoba-Aguilar and González-Tokman
102 2014; Kemp 2018; Grether 2019), and fights that do not immediately result in territory
103 turnover likely reduce the ability of the residents to win future fights. Thus, selection may
104 favor divergence in microhabitat use because this reduces the probability of interspecific
105 encounters and therefore the frequency of interspecific fights.

106 Species differences in microhabitat use have been documented in *Hetaerina*
107 (Johnson 1973; Anderson and Grether 2011), but it is unknown whether these differences

108 are products of past or ongoing interspecific aggression. Sympatric species could differ in
109 microhabitat use for reasons unrelated to interspecific aggression, or because one species
110 actively displaces the other from preferred microhabitat, or because of selection against
111 interspecific fighting, i.e., divergent ACD (Grether et al. 2009).

112 In this paper, we analyze data collected in the field over a 13-year period on 14
113 species pairs of rubyspot damselflies to examine whether current levels of interspecific
114 fighting can be explained by species pair differences in spatial segregation and species
115 discrimination, and to test the ACD prediction that species that respond more
116 aggressively to each other in direct encounters show higher levels of divergence in
117 microhabitat use. To the best of our knowledge, this is the first study, on any taxon, to
118 approach either of these questions with an adequate level of replication at the species
119 level.

120

121 **METHODS**

122 **Sympatric populations**

123 We studied 14 sympatric species pairs (10 different species) across 15 sites in the
124 southwestern United States, Mexico, and Costa Rica from 2005 to 2017 (see
125 Supplementary Methods S1 for criteria for inclusion of study sites). Some sites were
126 visited multiple times in different years. Because of interannual variation in microhabitat
127 availability and species densities, pooling the data across visits could have obscured
128 patterns of interest. We therefore kept visits to the same sites in different years separate

129 for the analyses presented in this paper, for a total of 25 species-pair-site-years, which we
130 refer to henceforth as sympatric population pairs (Table S1).

131

132 **Behavioral sampling**

133 At the beginning of each study period, we established a 200-300 m transect by fastening a
134 rope with numbered flags in 1-m increments along one or both banks of the river. Males
135 within the transect were captured with aerial nets, marked on the abdomen with unique
136 color codes using paint pens (200-S Fine Point, MarvyDecocolor Paint Marker; Uchida
137 of America, Torrance, CA, USA; Anderson et al. 2011), photographed (Figure S1), and
138 released where they were captured. Each day, 2-5 observers continuously walked along
139 the transect during periods of territorial activity (~0800-1800) recording the ID and
140 locations of males (as [x, y, z] coordinates where x is the flag number, y is the distance
141 from the bank of the stream, and z is the height) to the nearest 0.1 m on hand-held
142 computers (Psion PLC, London). In total, we marked 7,483 males and made 34,614
143 observations. A male was considered a territory holder if he was observed in the same
144 location (± 2 m) and perching close to the water, as males do when they are defending a
145 site, on at least two consecutive days (Anderson and Grether 2010a).

146 When fights were observed, we recorded the species involved, the males' IDs (if
147 marked), and the location. Fights between the same two marked males on the same day,
148 and fights involving unmarked males at the same location on the same day, were
149 collapsed into a single fight for the purpose of calculating intra- and interspecific fighting

150 frequencies (Anderson and Grether 2011; Drury and Grether 2014).

151

152 **Microhabitat sampling**

153 To quantify the microhabitat use of the damselflies at each site, we measured canopy
154 cover, stream width, stream current speed, and the perch height of territory holders. We
155 measured canopy cover, an especially important axis of microhabitat variation for
156 ectothermic insects (Shelly 1982; Huey 1991; Tsubaki et al. 2010; Okuyama et al. 2013),
157 every 5-10 m along the stream using a concave spherical densiometer (Forestry
158 Suppliers, Inc.). We made one measurement in the middle of the stream where the stream
159 was ≤ 3 m wide, two measurements (one on each side of the stream) where the stream was
160 3-10 m wide, and three measurements (one on each side of the stream and another in the
161 middle) where the stream was ≥ 10 m wide. Canopy cover ranges from 0 to 100% and
162 higher values indicate shadier habitat. We measured stream width every 2 m along the
163 transect with a measuring tape and visually assessed current speed every 2 m near both
164 banks and the middle of the stream using a 0 to 4 scale, where 0 is still water and 4 is
165 rapidly moving white water. To characterize the microhabitat of each male's territory, we
166 interpolated between the two nearest canopy cover, stream width, and current speed
167 readings, and averaged the male's recorded perch heights.

168

169 **Expected frequencies of interspecific fighting**

170 We considered interspecific fighting to be reduced relative to intraspecific fighting if the
171 observed frequency of interspecific fights was lower than expected based on a simple null

172 model (Anderson and Grether 2011). The null model assumes that males encounter and
173 fight with conspecific and heterospecific males in direct proportion to the species'
174 relative densities. The null expectation for the frequency of interspecific fights is simply
175 the observed total number of fights multiplied by $2d_i d_j$ where d_i and d_j are the species'
176 relative densities (Supplementary Methods S2a). All previous comparisons of observed
177 and expected rates of interspecific fighting in *Hetaerina* were based on this null model
178 (Anderson and Grether 2011; Drury et al. 2015).

179 Factors that could cause the observed frequency of interspecific fights to be lower
180 than the null expectation include: (1) spatial segregation between the species, which
181 would reduce the frequency of interspecific encounters; and (2) species discrimination,
182 which would reduce the probability of interspecific encounters resulting in territorial
183 fights. To evaluate whether spatial segregation alone accounts for the reduction in
184 interspecific fighting, we constructed lists of all males of each species observed within 4
185 m of the center of each established territory. From these lists, we calculated the average
186 proportion of heterospecific "neighbors" from each species' perspective and multiplied
187 the average of these two estimates by the total number of observed fights to obtain the
188 expected frequency of interspecific fights (Supplementary Methods S2b). The 4 m-
189 criterion is based on the observation that males respond to conspecific males up to ~2 m
190 away from their perch and in doing so could enter the reaction zone of a male perched 4
191 m away; beyond a distance of 4 m males are unlikely to interact (Anderson and Grether
192 2011 used the same criterion for similar reasons).

193 To evaluate whether species discrimination alone accounts for the reduction in
194 interspecific fighting, we calculated the expected interspecific fighting rate by
195 multiplying the null expectation by the probability of males chasing a heterospecific
196 intruder. Heterospecific chase probabilities were calculated from the results of simulated
197 territory intrusion tests in which territory holders were presented with live, tethered males
198 in timed trials (Anderson and Grether 2010a; Supplementary Methods S2c).

199 Finally, we combined the methods above to calculate the expected frequency of
200 interspecific fighting based on observed levels of spatial segregation and species
201 discrimination (Supplementary Methods S2d).

202

203 **Heterospecific aggression ratio**

204 The ACD hypothesis predicts that species pairs with high heterospecific aggression (HA)
205 will differ more in microhabitat use than those with low HA. We obtained a relative
206 measure of HA for each species in each sympatric population pair by dividing the
207 average proportion of time heterospecifics were chased by the average proportion of time
208 conspecifics were chased in the simulated territory intrusion trials, and refer to this as the
209 HA ratio (Grether et al. 2020; Table S2).

210

211 **Statistical methods**

212 We used chi-squared tests to determine whether observed frequencies of interspecific
213 fighting differed from expected frequencies, the Monte Carlo simulation method to
214 calculate p -values in cases with expected frequencies < 5 , and Holm's sequential

215 Bonferroni procedure (Holm 1979) to correct for multiple comparisons. Wilcoxon
216 matched pairs signed ranks tests were used to compare the alternative methods of
217 calculating expected frequencies to the null model.

218 To examine the causes of variation in interspecific fighting rates, we constructed a
219 linear multiple regression model where the dependent variable was the log of the ratio of
220 the observed number of interspecific fights to the expected number of fights under the
221 null model. The predictors were the mean proportion of heterospecific neighbors, the
222 species difference in microhabitat use, and the heterospecific chase probability ($n = 25$;
223 Supplementary Methods S2c Equation S2b).

224 We used principal component analysis (PCA) to find the principal axes of
225 variation in microhabitat use across all territory holders in the study ($n = 1974$). To obtain
226 an overall measure of the species difference in microhabitat use at each site, we
227 calculated the Euclidean distance between the species' PC centroids ($n = 25$).

228 To more fully characterize species differences in microhabitat use at each site, we
229 used linear discriminant analysis (LDA) and mixed-effects multivariate regression. The
230 microhabitat variables were transformed to a mean of 0 and variance of 1 to make the
231 LDA coefficients comparable and to weight the microhabitat variables equally in the
232 regression models. The predictor variables in the regression models were species (1 or 2),
233 an index identifying the microhabitat variable (1-4), the species by microhabitat variable
234 interaction, and a random-effects term for male ID ($n = 1974$). To make the sign of the
235 mean difference between species the same for all four microhabitat variables, we

236 assigned the species with the smaller mean an index of 1 and the species with the larger
237 mean an index of 2 (Table S3).

238 The ACD hypothesis predicts that species that respond more aggressively when
239 interspecific encounters occur should differ more in microhabitat use and exhibit higher
240 levels of spatial segregation. To test the first part of this prediction, we constructed linear
241 mixed-effects regression models with all sympatric population pairs included ($n = 25$)
242 and nested random-effects terms for population pair and male ID. The full, multivariate
243 model included indices to identify the species (1 or 2) and microhabitat variables (1-4),
244 the mean HA ratio, and all interactions between these terms. We also constructed
245 separate models for each microhabitat variable, with species index, mean HA ratio, the 2-
246 way interaction, and a random-effects term for sympatric population pair, and used the
247 Akaike information criterion (AIC) to evaluate whether adding quadratic terms improved
248 the model fit. We used a similar approach to test for effects of heterospecific aggression
249 on the proportion of heterospecifics in a male's territory neighborhood but coded the
250 species index based on the relative density of territory holders (1 = low, 2 = high).

251 Mixed-effects regression models were implemented with `mixed` in STATA 16.1
252 (StataCorp 2019). Other analyses were carried out in R 4.0.3 – 4.0.5 (R Core Team 2020,
253 2021); LDA was implemented with the `lda` default in R package MASS 7.3-53.1
254 (Venables and Ripley 2002).

255

256 **RESULTS**

257 **Species differences in microhabitat use**

258 The first three principal components (PCs) accounted for 83.4% of the variance in
259 microhabitat use (Table 1). PC1 explained 33.8% of the variance and had a large positive
260 loading for canopy cover and negative loadings for the other variables (Table 1; Figure
261 S2). PC2 explained 26.3% of the variance and had a large negative loading for current
262 speed and a positive loading for perch height, while PC3 explained 23.3% of the variance
263 and had a large positive loading for stream width and a negative loading for current speed
264 (Table 1; Figure S2). PC1 likely represents variation in stream size (smaller streams tend
265 to be slower and make smaller gaps in the forest canopy) while the other axes represent
266 variation in stream gradient and size independent of canopy cover (males tend to perch
267 low on emergent rocks in fast current and higher in the bank vegetation in slower
268 sections).

269 Twenty three of the 25 sympatric populations differ significantly in microhabitat
270 use (Table 2). Overall, the LDA correctly classified 79.7% of territory holders to species
271 based on microhabitat use, and for many populations the species classifications were 80-
272 100% correct (Table 2). As shown by the species means and LDA coefficients, all four
273 microhabitat variables proved useful for differentiating between sympatric species
274 (Tables 2, S3).

275

276 **Interspecific fighting**

277 Across the 25 pairs of sympatric populations, we collected data on 1,974 territory holders
278 and 1,793 fights, of which 346 (19.3%) were between heterospecific males. The observed
279 frequency of interspecific fights was significantly lower than the null expectation in 21

280 out of 25 cases (Table 3). There was considerable variation in this relationship across
281 species, as reflected by the wide range of chi-square values (Table 3). The multiple
282 regression analysis with species differences in neighborhood composition, microhabitat,
283 and chase probabilities as predictors accounted for 54% of the variation in the ratio of
284 observed to expected interspecific fights (Table 4). The greater the proportion of
285 heterospecifics in a territory holder's neighborhood and the greater the species difference
286 in microhabitat use, the lower the ratio of observed to expected interspecific fights.

287 We were also able to explain much of the reduction in the frequency of observed
288 interspecific fights compared to the null expectation. In some sympatric population pairs,
289 the reduction in the frequency of interspecific fights was explained by spatial segregation
290 (Figure 1a), while in others the reduction was explained by species discrimination (Figure
291 1b) or both spatial segregation and species discrimination (Figure 1c). Yet there were
292 some sympatric populations for which these factors could not fully explain the reduction
293 in observed interspecific fights (Figure 1d). The mean difference between the number of
294 observed and expected interspecific fights decreased by 32.4% when the expected rate
295 was calculated using only neighborhood composition, 19.1% using only chase
296 probabilities, and 50% with neighborhood composition and chase probabilities combined
297 (Table 5).

298

299 **Effects of interspecific aggression on microhabitat and spatial partitioning**

300 Overall, we found striking support for the hypothesis that interspecific aggression drives
301 species apart in microhabitat use. In the full multivariate model, the 3-way interaction

302 was highly significant ($\chi^2 = 85.70$, $df = 3$, $p < 0.0001$), which indicates that the effect of
303 heterospecific aggression on the species difference in microhabitat use varies strongly
304 among microhabitat variables. We therefore analyzed the microhabitat variables
305 separately. Adding quadratic terms substantially improved the fit of the perch height
306 ($\Delta AIC = -15.55$) and stream width ($\Delta AIC = -14.58$) models but worsened the fit of the
307 current speed ($\Delta AIC = 3.25$) and canopy cover models ($\Delta AIC = 0.26$). The species
308 difference in perch height was greater between sympatric populations with low and high
309 HA compared to those with intermediate HA (Figure 2; species \times HA: -0.68 ± 0.14 , $z = -$
310 4.91 , $p < 0.0001$; species \times HA²: 0.43 ± 0.11 , $z = 3.96$, $p = 0.001$). The species difference
311 in the other three microhabitat variables increased with the HA ratio (Figure 2; stream
312 width, species \times HA: -4.64 ± 1.88 , $z = -2.47$, $p = 0.013$; species \times HA²: 5.95 ± 1.45 , $z =$
313 4.11 , $p < 0.0001$; current speed, species \times HA: 0.16 ± 0.074 , $z = 2.20$, $p = 0.028$; canopy
314 cover, species \times HA: 15.31 ± 2.28 , $z = 6.70$, $p < 0.0001$). Also as predicted by the ACD
315 hypothesis, the proportion of heterospecific neighbors decreased, and thus spatial
316 segregation increased, with the level of heterospecific aggression (Figure 3; HA: $-0.20 \pm$
317 0.06 , $z = -3.22$, $p = 0.001$), particularly for species with a low relative density of territory
318 holders, as indicated by a positive interaction between the relative density of territory
319 holders and the HA ratio (0.095 ± 0.035 , $z = 2.72$, $p = 0.0066$).

320 To evaluate whether the results were affected by males at site GO01 contributing
321 data to two different sympatric population pairs, we ran the mixed-effects regression
322 models on subsets of the data and found that dropping any two GO01 pairs had no
323 qualitative effect on the results (Table S4).

324

325 **DISCUSSION**

326 This 13-year investigation of 14 species pairs provides an unprecedented level of support
327 for the general hypothesis that interspecific aggression increases spatial habitat
328 partitioning between sympatric species. Specifically, we found that sympatric species that
329 are more aggressive to each other in simulated intruder tests differ more in microhabitat
330 use (Figure 2) and are more spatially segregated (Figure 3). In principle, three non-
331 mutually exclusive mechanisms could have produced this pattern: species sorting,
332 competitive displacement, and agonistic character displacement (ACD). We discuss each
333 of these potential mechanisms in turn and explain why we consider ACD to be the most
334 likely mechanism.

335 In this context, species sorting refers to effects of interspecific interactions on the
336 probability of species occurring in sympatry (Pfennig and Pfennig 2012). If interspecific
337 fighting reduces the probability of co-occurrence, the positive relationship between
338 microhabitat partitioning and heterospecific aggression could be a byproduct of variation
339 in the level of microhabitat divergence prior to secondary contact. It has yet to be shown,
340 however, that interspecific fighting affects the probability of co-occurrence in
341 damselflies. Most research on coexistence mechanisms in Odonata has focused on
342 resource competition and predation at the larval stage (e.g., McPeck 2004; Siepielski et
343 al. 2010; Siepielski et al. 2011; Bried and Siepielski 2019); it is not yet clear whether
344 behavioral interference at the adult stage affects coexistence in this taxon (reviewed in
345 Grether et al. 2022).

346 Competitive displacement has been shown, or strongly inferred, to be the primary
347 cause of species differences in habitat use in other territorial animals, including insects
348 and arachnids (Reitz and Trumble 2002), barnacles (Connell 1961), mammals (Brown
349 1971; Pasch et al. 2013), birds (Garcia 1983; Jankowski et al. 2010; Kajtoch et al. 2015;
350 Martin and Bonier 2018), and reptiles (Langkilde and Shine 2004; Edgehouse et al.
351 2014). A common feature of systems in which competitive displacement occurs is that
352 one species is competitively superior and displaces the other species from the preferred
353 habitat (Reitz and Trumble 2002). In general, there are several ways that one species
354 could be competitively superior, but in the case of damselflies competing for mating
355 territories, competitive superiority would entail behavioral dominance or superior aerial
356 fighting ability. We are not aware of any rubyspot damselfly species pairs in which one
357 species is dominant or consistently wins territorial fights, but further research is
358 warranted. Whether competitive displacement occurs, and the extent to which it explains
359 the effects of heterospecific aggression on microhabitat use, could be tested with removal
360 experiments or microhabitat manipulations.

361 While species sorting and competitive displacement are both plausible post-hoc
362 explanations, neither of those hypotheses could have been used to predict that
363 microhabitat partitioning would correlate positively with heterospecific aggression
364 without making unsupported assumptions about the study system. By contrast this was a
365 well-founded prediction of the agonistic character displacement hypothesis. Previous
366 research showed that some sympatric rubyspot damselfly species have diverged
367 substantially in male wing coloration and competitor recognition, and that the territories

368 of these species often overlap extensively (Anderson and Grether 2010a; Anderson and
369 Grether 2010b; Anderson and Grether 2011). In most rubyspot damselfly species pairs,
370 however, interspecific territorial aggression is adaptive because females of these species
371 are too similar in coloration for males to distinguish between them; a territory holder that
372 tolerated heterospecific males on his territory would risk losing mating opportunities
373 (Drury, Okamoto, et al. 2015; Drury et al. 2019; Grether et al. 2020). In this situation,
374 divergence in microhabitat preferences might be the only way for selection to reduce the
375 costs of interspecific aggression. Our initial evidence that microhabitat divergence has
376 evolved in response to interspecific aggression was based on four sympatric species pairs
377 (Anderson and Grether 2011). Now with data on 14 sympatric species pairs, across
378 multiple sites and years, we can confirm that microhabitat divergence is strongly
379 associated with interspecific aggression (Figure 2).

380 We expect positive relationships between habitat partitioning and heterospecific
381 aggression to be found in other taxa as well. Our other findings are rather damselfly
382 specific, but parallels might be found in other taxa. For example, interspecifically
383 territorial birds are expected to vertically stratify in habitats with a large height
384 dimension, such as woodlands (Murray 1971). Indeed lunulated and Salvin's antbirds
385 (*Gymnopithys lunulate* and *G. salvini*) forage from taller perches in the presence of
386 larger, behaviorally dominant antbirds and woodcreepers (Willis 1968). Similarly, the
387 iguanid lizard *Liolaemus tenuis* perches higher when sympatric with the aggressively
388 dominant *L. pictus* (Medel et al. 1988). Rubyspot damselfly species with both low and
389 high levels of heterospecific aggression differ more in mean perch height than those with

390 intermediate levels of heterospecific aggression (Figure 2). Considering that species with
391 low levels of heterospecific aggression have overlapping territories (Anderson & Grether
392 2011), the species differences in perch height probably function to reduce accidental
393 interspecific interference.

394 We found a negative relationship, at the population level, between the mean
395 proportion of heterospecific neighbors and the ratio of observed to expected frequencies
396 of interspecific fights (Table 4). Logically, territory holders with more heterospecific
397 neighbors should be observed in more, not fewer, interspecific fights. The
398 counterintuitive population-level result is probably an artifact of the mathematical
399 constraint that males in populations with lower relative densities have more
400 heterospecific neighbors. What this population-level analysis did show, however, is that
401 species differences in microhabitat use reduce interspecific fighting (Table 4).

402 When we based expected frequencies on the proportion of heterospecific
403 neighbors and the probability of males responding aggressively to heterospecifics, the
404 mean difference between the observed and expected numbers of interspecific fights was
405 50% less than under the null model (Table 5). However, the observed number of
406 interspecific fights was still significantly below the expected number in many populations
407 (Figures 1, S3). Species differences in microhabitat use, which were found in all but two
408 sympatric population pairs (Table 2), likely reduce the frequency of interspecific fights
409 below what would be expected based on the composition of territorial neighborhoods and
410 heterospecific aggression. For example, species that perch at different heights tend to
411 fight at different heights (authors, pers. obs.), and therefore may be less likely to fight

412 with heterospecific neighbors than expected based on the spatial arrangement of
413 territories. Additionally, differences between species in stream current speed preferences
414 might also reduce the frequency of interspecific fighting because current speed can vary
415 among neighboring territories.

416 Studies on other taxa have also revealed adaptive connections between
417 interspecific aggression and microhabitat use. For example, fine-scale microhabitat
418 partitioning has been reported in interspecifically territorial damselfish (Eurich et al.
419 2018). Territorial neotropical cichlid fish (*Amphilophilous* spp.) are more likely to
420 tolerate heterospecific neighbors with divergent coloration (Lehtonen et al. 2010;
421 Lehtonen et al. 2015). Interspecifically aggressive nightingales (*Luscinia megarhynchos*
422 and *L. luscinia*) “escape” to allotopic sites in the sympatric region of their geographic
423 ranges and occupy habitat avoided by the congener (Reif et al. 2018).

424 Selection against interspecific interference is only one of many possible reasons
425 that closely related species might differ in microhabitat preferences. For example,
426 microhabitat preferences could have diverged in allopatry before secondary contact
427 (Berner and Thibert-Plante 2015; Dufour et al. 2015). Conspecific attraction might also
428 reduce spatial overlap between sympatric species (Scott and Lee 2013; Stodola and Ward
429 2017) and result in chance differences in microhabitat use (Buxton et al. 2020).

430 Nevertheless, the results presented here provide compelling evidence that interspecific
431 aggression has played an important role in microhabitat divergence.

432

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450

451 **DATA ACCESSIBILITY**

452 Analyses reported in this article can be reproduced using the data provided by McEachin et
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454 **REFERENCES**

- 455 Anderson CN, Cordoba-Aguilar A, Drury JP, Grether GF. 2011. An assessment of
456 marking techniques for odonates in the family Calopterygidae. *Entomol Exp Appl.*
457 141(3):258–261.
- 458 Anderson CN, Grether GF. 2010a. Interspecific aggression and character displacement of
459 competitor recognition in *Hetaerina* damselflies. *Proc R Soc B Biol Sci.* 277:549–
460 555.
- 461 Anderson CN, Grether GF. 2010b. Character displacement in the fighting colours of
462 *Hetaerina* damselflies. *Proc R Soc B Biol Sci.* 277:3669–3675.
- 463 Anderson CN, Grether GF. 2011. Multiple routes to reduced interspecific territorial
464 fighting in *Hetaerina* damselflies. *Behav Ecol.* 22(3):527–534.
- 465 Bay LK, Jones GP, McCormick MI. 2001. Habitat selection and aggression as
466 determinants of spatial segregation among damselfish on a coral reef. *Coral Reefs.*
467 20:289–298.
- 468 Berner D, Thibert-Plante X. 2015. How mechanisms of habitat preference evolve and
469 promote divergence with gene flow. *J Evol Biol.* 28(9):1641–1655.
- 470 Bried JT, Siepielski AM. 2019. Predator driven niches vary spatially among co-occurring
471 damselfly species. *Evol Ecol.* 33(2):243–256.
- 472 Brown JH. 1971. Mechanisms of competitive exclusion between two species of
473 chipmunks. *Ecology.* 52(2):305–311.
- 474 Buxton VL, Enos JK, Sperry JH, Ward MP. 2020. A review of conspecific attraction for
475 habitat selection across taxa. *Ecol Evol.* 10(23):12690–12699.

476 Cody ML. 1973. Character convergence. *Annu Rev Ecol Syst.* 4:189–211.

477 Connell JH. 1961. The influence of interspecific competition and other factors on the
478 distribution of the barnacle *Chthamalus stellatus*. *Ecology.* 42(4):710–723.

479 Connell JH. 1978. Diversity in tropical rain forests and coral reefs. *Science.*
480 199(4335):1302–1310.

481 Connell JH. 1983. On the prevalence and relative importance of interspecific
482 competition: Evidence from field experiments. *Am Nat.* 122(5):661–696.

483 Córdoba-Aguilar A, González-Tokman DM. 2014. The behavioral and physiological
484 ecology of adult rubyspot damselflies (*Hetaerina*, Calopterygidae: Odonata). In:
485 *Advances in the Study of Behavior.* Vol. 46. Academic Press Inc. p. 311–341.

486 Córdoba-Aguilar A, Raihani G, Serrano-Meneses MA, Contreras-Garduno J. 2009. The
487 lek mating system of *Hetaerina* damselflies (Insecta: Calopterygidae). *Behaviour.*
488 146:189–207.

489 Cowen MC, Drury JP, Grether GF. 2020. Multiple routes to interspecific territoriality in
490 sister species of North American perching birds. *Evolution.* 74(9):2134–2148.

491 Drury JP, Anderson CN, Castillo MC, Fisher J, McEachin S, Grether GF. 2019. A
492 general explanation for the persistence of reproductive interference. *Am Nat.*
493 194(2):268–275.

494 Drury JP, Anderson CN, Grether GF. 2015. Seasonal polyphenism in wing coloration
495 affects species recognition in rubyspot damselflies (*Hetaerina* spp.). *J Evol Biol.*
496 28(8):1439–1452.

497 Drury JP, Grether GF. 2014. Interspecific aggression, not interspecific mating, drives

498 character displacement in the wing coloration of male rubyspot damselflies
499 (*Hetaerina*). Proc R Soc B Biol Sci. 281(1796).

500 Drury JP, Okamoto KW, Anderson CN, Grether GF. 2015. Reproductive interference
501 explains persistence of aggression between species. Proc R Soc B Biol Sci.
502 282:20142256.

503 Dufour CMS, Meynard C, Watson J, Rioux C, Benhamou S, Perez J, du Plessis JJ,
504 Avenant N, Pillay N, Ganem G. 2015. Space use variation in co-occurring sister
505 species: Response to environmental variation or competition? PLoS One.
506 10(2):e0117750.

507 Edgehouse M, Latta IV LC, Brodie ED, Brodie ED. 2014. Interspecific aggression and
508 habitat partitioning in garter snakes. PLoS One. 9(1):1–5.

509 Eurich JG, McCormick MI, Jones GP. 2018. Habitat selection and aggression as
510 determinants of fine-scale partitioning of coral reef zones in a guild of territorial
511 damselfishes. Mar Ecol Prog Ser. 587:201–215.

512 Garcia EFJ. 1983. An experimental test of competition for space between blackcaps
513 *Sylvia atricapilla* and garden warblers *Sylvia borin* in the breeding season. J Anim
514 Ecol. 52(3):795–805.

515 Garrison RW. 1990. A synopsis of the genus *Hetaerina* with descriptions of four new
516 species (Odonata: Calopterygidae). Trans Am Entomol Soc. 116(1):175–259.

517 Gotelli NJ, Graves GR, Rahbek C. 2010. Macroecological signals of species interactions
518 in the Danish avifauna. PNAS. 107(11):5030–5035.

519 Grether GF. 1996. Intrasexual competition alone favors a sexually dimorphic ornament in

520 the rubyspot damselfly *Hetaerina americana*. *Evolution*. 50(5):1949–1957.

521 Grether GF. 2019. Territoriality in aquatic insects. In: *Aquatic Insects*. Springer
522 International Publishing. p. 167–189.

523 Grether GF, Anderson CN, Drury JP, Kirschel ANG, Losin N, Okamoto K, Peiman KS.
524 2013. The evolutionary consequences of interspecific aggression. *Ann N Y Acad*
525 *Sci*. 1289(1):48–68.

526 Grether GF, Drury JP, Berlin E, Anderson CN. 2015. The role of wing coloration in sex
527 recognition and competitor recognition in rubyspot damselflies (*Hetaerina* spp.).
528 *Ethology*. 121(7):674–685.

529 Grether GF, Drury JP, Okamoto KW, McEachin S, Anderson CN. 2020. Predicting
530 evolutionary responses to interspecific interference in the wild. *Ecol Lett*.
531 23(2):221–230.

532 Grether GF, Losin N, Anderson CN, Okamoto K. 2009. The role of interspecific
533 interference competition in character displacement and the evolution of competitor
534 recognition. *Biol Rev*. 84(4):617–635.

535 Grether GF, Peiman KS, Tobias JA, Robinson BW. 2017. Causes and consequences of
536 behavioral interference between species. *Trends Ecol Evol*. 32(10):760–772.

537 Grether GF, Siepielski AM, Gomez-Llano MA. 2022. Ecological differentiation,
538 interference, and coexistence in odonates. In: Cordoba-Aguilar A, Beatty CD, Bried
539 JT, Suarez-Tovar CM, editors. *Dragonflies and damselflies: Model organisms for*
540 *ecological and evolutionary research*, Second Edition. Forthcoming.

541 Guillermo-Ferreira R, Del-Claro K. 2011. Resource defense polygyny by *Hetaerina*

542 *rosea* Selys (Odonata: Calopterygidae): Influence of age and wing pigmentation.
543 Neotrop Entomol. 40(1):78–84.

544 Hawkins A. 1999. Altitudinal and latitudinal distribution of east Malagasy forest bird
545 communities. J Biogeogr. 26:447–458.

546 Holm S. 1979. A simple sequentially rejective multiple test procedure. Scand J Ornithol.
547 6(2):65-70.

548 Huey RB. 1991. Physiological consequences of habitat selection. Am Nat. 137:S91–
549 S115.

550 Jankowski JE, Robinson SK, Levey DJ. 2010. Squeezed at the top: Interspecific
551 aggression may constrain elevational ranges in tropical birds. Ecology. 91(7):1877–
552 1884.

553 Johnson C. 1963. Interspecific territoriality in *Hetaerina americana* (Fabricius) and *H.*
554 *titia* (Drury) (Odonata: Calopterygidae) with a preliminary analysis of the wing
555 color pattern variation. Can Entomol. 95:575–582.

556 Johnson C. 1966. Environmental modification of habitat selection in adult damselflies.
557 Ecol Soc Am. 47(4):674–676.

558 Johnson C. 1973. Distributional patterns and their interpretation in *Hetaerina* (Odonata:
559 Calopterygidae). Florida Entomol. 56(1):24–42.

560 Kajtoch Ł, Żmihorski M, Wieczorek P. 2015. Habitat displacement effect between two
561 competing owl species in fragmented forests. Popul Ecol. 57(3):517–527.

562 Kirschel ANG, Seddon N, Tobias JA. 2019. Range-wide spatial mapping reveals
563 convergent character displacement of bird song. Proc R Soc B Biol Sci.

564 286(1902):20190443.

565 Korner P, Whiting MJ, Willem J, Ferguson H. 2010. Interspecific aggression in flat
566 lizards suggests poor species recognition. *African J Herpetol.* 49(2):139–146.

567 Langkilde T, Shine R. 2004. Competing for crevices: Interspecific conflict influences
568 retreat-site selection in montane lizards. *Oecologia.* 140:684–691.

569 Latour Y, Ganem G. 2017. Does competitive interaction drive species recognition in a
570 house mouse secondary contact zone? *Behav Ecol.* 28(1):212–221.

571 Lehtonen TK, McCrary JK, Meyer A. 2010. Territorial aggression can be sensitive to the
572 status of heterospecific intruders. *Behav Processes.* 84(2):598–601.

573 Lehtonen TK, Sowersby W, Gagnon K, Wong BBM. 2015. Cichlid fish use coloration as
574 a cue to assess the threat status of heterospecific intruders. *Am Nat.* 186(4):547–552.

575 Lewin R. 1989. Biologists disagree over bold signature of nature. *Science.*
576 244(4904):527–528.

577 Mark AF, Dickinson KJM, Allen J, Smith R, West CJ. 2001. Vegetation patterns, plant
578 distribution and life forms across the alpine zone in southern Tierra del Fuego,
579 Argentina. *Austral Ecol.* 26(4):423–440.

580 Martin PR, Bonier F. 2018. Species interactions limit the occurrence of urban-adapted
581 birds in cities. *PNAS.* 115(49):E11495–E11504.

582 McEachin S, Drury JP, Anderson CN, Grether GF. 2001. Data from: Mechanisms of
583 reduced interspecific interference between territorial species. *Behavioral Ecology.*
584 <https://doi.org/10.5068/D16Q39>.

585 McPeck MA. 2004. The growth/predation risk trade-off: So what is the mechanism? *Am*

586 Nat. 163(5):E88-111.

587 Medel RG, Marquet PA, Jaksic FM. 1988. Microhabitat shifts of lizards under different
588 contexts of sympatry: A case study with South American *Liolaemus*. *Oecologia*.
589 76:567-569.

590 Melville J. 2002. Competition and character displacement in two species of scincid
591 lizards. *Ecol Lett*. 5:386–393.

592 Moran RL, Fuller RC. 2018a. Male-driven reproductive and agonistic character
593 displacement in darters and its implications for speciation in allopatry. *Curr Zool*.
594 64(1):101–113.

595 Moran RL, Fuller RC. 2018b. Agonistic character displacement of genetically based male
596 colour patterns across darters. *Proc R Soc B Biol Sci*. 285:20181248.

597 Murray BG. 1971. The ecological consequences of interspecific territorial behavior in
598 birds. *Ecol Soc Am*. 52(3):414–423.

599 Nishikawa KC. 1987. Interspecific aggressive behaviour in salamanders: Species-specific
600 interference or misidentification? *Anim Behav*. 35:263–270.

601 Okuyama H, Samejima Y, Tsubaki Y. 2013. Habitat segregation of sympatric *Mnais*
602 damselflies (Odonata: Calopterygidae): Microhabitat insolation preferences and
603 competition for territorial space. *Int J Odonatol*. 16(2):109–117.

604 Pasch B, Bolker BM, Phelps SM. 2013. Interspecific dominance via vocal interactions
605 mediates altitudinal zonation in neotropical singing mice. *Am Nat*. 182(5):E161–
606 E173.

607 Pasch B, Sanford R, Phelps SM. 2017. Agonistic character displacement in social

608 cognition of advertisement signals. *Anim Cogn.* 20(2):267–273.

609 Pfennig KS, Pfennig DW. 2012. *Evolution's wedge: Competition and the origins of*
610 *diversity.* University of California Press.

611 Pigot AL, Tobias JA. 2013. Species interactions constrain geographic range expansion
612 over evolutionary time. *Ecol Lett.* 16(3):330–338.

613 Pinter-Wollman N, Dayan T, Eilam D, Kronfeld-Schor N. 2006. Can aggression be the
614 force driving temporal separation between competing common and golden spiny
615 mice? *J Mammal.* 87(1):48–53.

616 Randall JA. 1978. Behavioral mechanisms of habitat segregation between sympatric
617 species of *Microtus*: Habitat preference and interspecific dominance. *Behav Ecol*
618 *Sociobiol.* 3(2):187–202.

619 Reif J, Jiran M, Reifov R, Vokurkov J, Dolata PT, Petrusek A, Petruskov T. 2015.
620 Interspecific territoriality in two songbird species: Potential role of song
621 convergence in male aggressive interactions. *Anim Behav.* 104:131–136.

622 Reif J, Reifová R, Skoracka A, Kuczyński L. 2018. Competition-driven niche segregation
623 on a landscape scale: Evidence for escaping from syntopy towards allotopy in two
624 coexisting sibling passerine species. *J Anim Ecol.* 87(3):774–789.

625 Reitz SR, Trumble JT. 2002. Competitive displacement among insects and arachnids.
626 *Annu Rev Entomol.* 47(1):435–465.

627 Robinson SK, Terborgh J. 1995. Interspecific aggression and habitat selection by
628 Amazonian birds. *J Anim Ecol.* 64(1):1–11.

629 Ross ST. 1986. Resource partitioning in fish assemblages: A review of field studies. *Am*

630 Soc Ichthyol Herpetol. 1986(2):352–388.

631 Schoener TW. 1983. Field experiments on interspecific competition. Am Nat.
632 122(2):240–285.

633 Schultz JK, Switzer P V. 2001. Pursuit of heterospecific targets by territorial amberwing
634 dragonflies (*Perithemis tenera* Say): A case of mistaken identity. J Insect Behav.
635 14(5):607–620.

636 Scott TA, Lee P-Y. 2013. Reconciling social interaction with habitat selection in
637 territorial species. Theory Biosci. 132:41–46.

638 Shelly TE. 1982. Comparative foraging behavior of light-versus shade-seeking adult
639 damselflies in a lowland neotropical forest (Odonata: Zygoptera). Physiol Zool.
640 55(4):335–343.

641 Siepielski AM, Hung K-L, Bein EEB, McPeck MA. 2010. Experimental evidence for
642 neutral community dynamics governing an insect assemblage. Ecology. 91(3):847–
643 857.

644 Siepielski AM, Mertens AN, Wilkinson BL, McPeck MA. 2011. Signature of ecological
645 partitioning in the maintenance of damselfly diversity. J Anim Ecol. 80(6):1163–
646 1173.

647 Singer F. 1989. Interspecific aggression in *Leucorrhinia* dragonflies: A frequency-
648 dependent discrimination threshold hypothesis. Behav Ecol Sociobiol. 25:421–427.

649 Souriau A, Kohoutová H, Reif J, Vokurková J, Petrusek A, Reifová R, Petrusková T.
650 2018. Can mixed singing facilitate coexistence of closely related nightingale
651 species? Behav Ecol. 29(4):925–932.

652 Stodola KW, Ward MP. 2017. The emergent properties of conspecific attraction can limit
653 a species' ability to track environmental change. *Am Nat.* 189(6):726–733.

654 Suhonen J, Rantala MJ, Honkavaara J. 2008. Territoriality in odonates. In: Córdoba-
655 Aguilar A, editors. *Dragonflies and damselflies: Model organisms for ecological and*
656 *evolutionary research.* Oxford Univ Press, Oxford, p. 203–218.

657 Tsubaki Y, Samejima Y, Siva-Jothy MT. 2010. Damselfly females prefer hot males:
658 Higher courtship success in males in sunspots. *Behav Ecol Sociobiol.* 64(10):1547–
659 1554.

660 Ulrich W, Jabot F, Gotelli NJ. 2017. Competitive interactions change the pattern of
661 species co-occurrences under neutral dispersal. *Oikos.* 126(1):91–100.

662 Vallin N, Rice AM, Bailey RI, Husby A, Qvarnström A. 2012. Positive feedback
663 between ecological and reproductive character displacement in a young avian hybrid
664 zone. *Evolution.* 66(4):1167–1179.

665 Vieira MC, Peixoto PEC. 2013. Winners and losers: A meta-analysis of functional
666 determinants of fighting ability in arthropod contests. *Funct Ecol.* 27(2):305–313.

667 Willis EO. 1968. Studies of the behavior of lunulated and Salvin's antbirds. *Condor.*
668 70(2):128–148.

669 Wisheu IC. 1998. How organisms partition habitats: Different types of community
670 organization can produce identical patterns. *Oikos.* 83(2):246–258.

671 Zambre AM, Khandekar A, Sanap R, O'Brien C, Snell-Rood EC, Thaker M. 2020.
672 Asymmetric interspecific competition drives shifts in signalling traits in fan-throated
673 lizards. *Proc R Soc B Biol Sci.* 287(1940):20202141.

674

675

676 **FIGURE LEGENDS**

677 **Figure 1.** Observed and expected numbers of interspecific fights, based on different
678 methods of calculating the expectation, for selected sympatric population pairs (see Fig.
679 S3 for the remaining populations). An example of the reduction in the number of
680 observed interspecific fights that can be explained by (a) spatial segregation of territory
681 holders based on the proportion of heterospecific males in territory holders'
682 neighborhoods (*H. occisa*-*H. miniata* at LH03 2016), (b) competitor recognition based on
683 the aggressive response of territory holders to heterospecific intruders relative to
684 conspecific intruders in simulated territory intrusions (*H. occisa*-*H. titia* at OT 2007), (c)
685 both spatial segregation and competitor recognition (*H. fuscoguttata*-*H. titia* at GO01
686 2016), and (d) a population pair in which neither spatial distribution nor competitor
687 recognition can account for the reduction in observed interspecific fights (*H. cruentata*-
688 *H. majuscula* at MV04 2016). Combined refers to the model that calculates expected
689 fighting rates based on both spatial segregation and competitor recognition. See Table 3
690 for statistical results comparing all 25 sympatric population pairs.

691

692 **Figure 2.** Evidence that interspecific aggression causes species to diverge in
693 microhabitat preferences. As heterospecific aggression increases, so do species
694 differences in territory microhabitat. The exception is perch height, which differs the least
695 between sympatric species at intermediate levels of heterospecific aggression. Points and
696 bars represent population means and standard errors. Triangles (circles) represent the

697 population with higher (lower) relative density in each pair. Colors uniquely identify the
698 paired populations. Lower (upper) black lines represent predicted values for the
699 populations with lower (higher) means of the corresponding microhabitat variable, and
700 gray areas are 95% confidence intervals, from the mixed-effects regression model
701 described in **Statistical methods**.

702

703 **Figure 3.** Evidence that interspecific aggression increases spatial separation between
704 species. The proportion of heterospecific neighbors decreases as heterospecific
705 aggression increases. The slope of the relationship is steeper for populations with low
706 relative density compared to those with high relative density. Lower (upper) black lines
707 represent predicted values for the populations with lower (higher) relative density in each
708 pair. All other symbols and codes follow Figure 2.

709

710

711 **Table 1** Microhabitat principal component loadings (% variance explained)

	PC1 (33.8%)	PC2 (26.3%)	PC3 (23.3%)	PC4 (16.6%)
Perch height	-0.579	0.522	-0.014	-0.626
Stream width	-0.345	-0.380	0.858	-0.017
Current speed	-0.284	-0.760	-0.459	-0.361
Canopy cover	0.682	-0.066	0.231	-0.691

712

713

714 **Table 2** Results of linear discriminant analyses (LDA) and multivariate regression

715 analyses (MVA) of species differences in microhabitat use

Pop. pair	Spp. code	LDA species classif.			LDA coefficients (standardized)				MVA
		# correct	# wrong	% correct	Perch height	Stream width	Current speed	Canopy cover	z
1	c	41	9	82	-0.31	-0.07	-1.40	0.50	7.62***
	o	33	5	87					
2	o	45	10	82	0.77	0.10	-0.30	0.23	4.83***
	t	17	12	59					
3	o	11	27	29	0.66	-0.02	-0.94	-0.30	2.23*
	t	44	10	81					
4	a	15	14	52	0.79	-0.20	-1.10	0.70	5.70***
	t	68	3	96					
5	a	38	9	81	0.78	-0.19	-1.17	0.66	4.82***
	t	18	16	53					
6	a	14	4	78	0.98	-0.03	0.06	-0.18	2.64**
	t	24	3	89					
7	a	48	8	86	-0.10	-0.01	0.65	1.06	5.85***
	c	23	4	85					
8	c	54	0	100	0.75	0.64	0.87	2.48	1.20
	v	0	15	0					
9	a	92	5	95	1.36	-0.04	-0.11	0.87	4.88***
	v	8	19	30					
10	o	120	19	86	2.17	-0.15	-0.09	0.32	5.61***
	t	38	38	50					
11	o	190	12	94	2.36	-0.11	-0.18	0.39	6.37***
	t	27	31	47					
12	o	46	3	94	1.73	-0.03	0.23	2.82	4.07***
	s	12	10	55					
13	o	16	3	84	-0.14	-0.95	0.17	6.15	2.22*
	p	9	3	75					
14	n	6	0	100	-1.03	0.20	-0.16	-0.85	14.65***
	o	80	2	98					
15	c	10	18	36	1.13	0.59	-0.76	-1.16	3.21**
	m	64	4	94					

16	c	28	8	78	0.83	0.35	0.53	2.14	6.32***
	p	34	0	100					
17	o	29	18	62	2.29	0.01	0.10	4.98	2.45*
	p	42	10	81					
18	n	8	8	50	2.32	0.03	-0.45	-1.48	2.35*
	t	48	2	96					
19	n	19	3	86	-0.28	0.11	-0.03	-0.61	6.34***
	o	41	12	77					
20	f	6	1	86	-2.25	0.39	1.79	-0.83	3.11*
	o	10	0	100					
21	f	4	3	57	0.51	-0.20	-0.50	-0.85	3.81***
	t	19	1	95					
22	o	8	2	80	0.80	-0.42	-1.02	-0.29	3.98***
	t	20	0	100					
23	f	16	2	89	-1.50	0.06	0.30	-2.19	2.38*
	o	11	6	65					
24	f	18	0	100	0.45	-0.24	-0.03	-2.48	1.27
	t	4	6	40					
25	o	13	4	76	1.87	-0.23	-0.49	0.66	2.35*
	t	6	4	60					

716 See Table S1 for site info and species names.

717 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

718

719

720 **Table 3** Comparison of the observed frequencies of interspecific fights to those expected

721 under the null model

Pop. pair	Spp.	Observed freq.		Expected freq.		Chi-sq. test
		Intrasp. fights	Intersp. fights	Intrasp. fights	Intersp. fights	
1	c	8	2	2.8	9.0	15.78**
	o	9		7.3		
2	o	13	6	19.4	25.3	88.18****
	t	34		8.3		
3	o	8	4	8.6	26.3	42.00****
	t	43		20.1		
4	a	6	8	2.4	18.6	12.63*
	t	43		36.0		
5	a	25	18	19.1	30.1	9.44*
	t	18		11.9		
6	a	3	3	3.5	18.7	23.86**
	t	41		24.8		
7	a	37	7	31.0	19.0	17.91*
	c	9		2.9		
8	c	38	23	39.9	23.5	4.46
	v	7		3.4		
9	a	31	12	28.5	16.2	2.57
	v	4		2.3		
10	o	16	11	15.6	21.2	17.47**
	t	17		7.2		
11	o	30	14	19.2	41.0	35.55****
	t	38		21.8		
12	o	26	12	17.5	26.5	15.61***
	s	16		10.0		
13	o	25	5	15.4	19.5	20.57****
	p	11		6.2		
14	n	2	2	2.2	25.5	29.25**
	o	98		74.3		
15	c	29	26	12.6	60.2	45.36****
	m	90		72.2		
16	c	27	15	4.5	33.8	123.95**

	p	60		63.7		
17	o	42	24	32.8	69.9	69.05***
	p	74		37.2		
18	n	7	33	13.2	75.8	49.12***
	t	158		109.0		
19	n	4	6	4.2	19.2	17.36**
	o	35		21.6		
20	f	13	7	10.2	10.2	1.85
	o	3		2.6		
21	f	13	18	10.1	36.7	17.81**
	t	49		33.2		
22	o	3	4	15.4	27.9	134.86***
	t	49		12.7		
23	f	145	53	125.5	100.4	64.22***
	o	48		20.1		
24	f	145	18	132.7	35.0	18.94*
	t	7		2.3		
25	o	48	15	39.6	26.1	8.20
	t	7		4.3		

722 See Table S1 for site info.

723 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after sequential Bonferroni correction

724

725 **Table 4** Predictors of variation in the ratio of observed to expected interspecific fights
726 under the null model

Predictor	B	SE	<i>p</i>
Intercept	0.01	0.24	0.98
Neighborhood composition	-1.08	0.39	0.011
Microhabitat differences	-0.30	0.058	<0.001
Chase probabilities	0.29	0.15	0.078

727 Linear multiple regression, $n = 25$, model adjusted $R^2 = 0.54$, $F(3, 21) = 10.4$, $p < 0.001$.

728

729

730 **Table 5** Comparison of three alternative methods of calculating expected frequencies of

731 interspecific fighting to the null model, with Wilcoxon matched pairs signed ranks tests

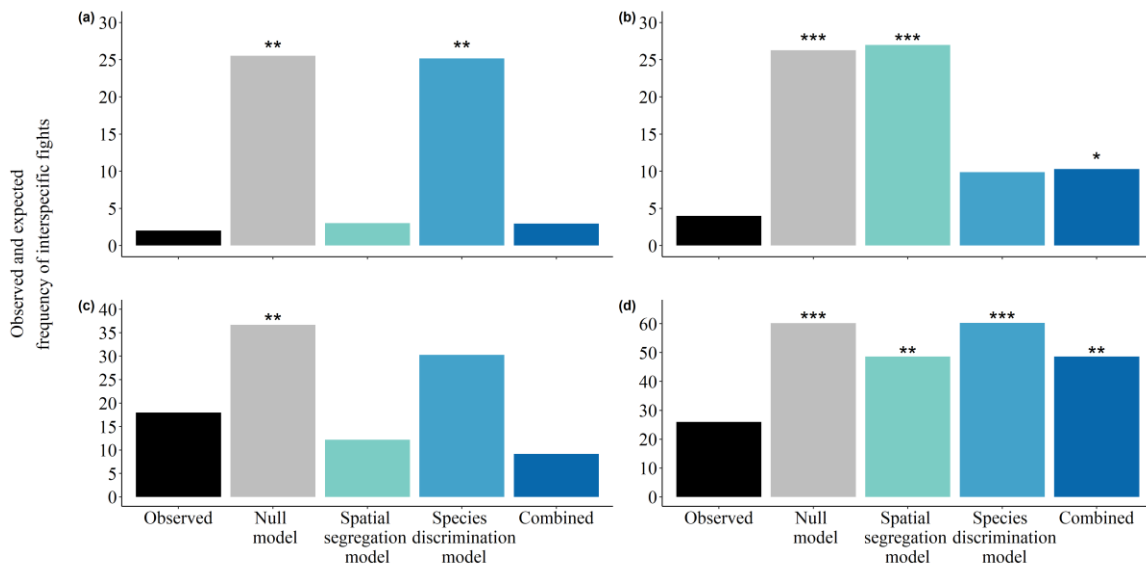
Model	Obs. – Exp.			<i>p</i>
	Mean	SE	V	
Relative density (null)	-18.8	2.52	-	-
Spatial segregation	-12.7	2.33	57	0.0034
Species discrimination	-15.2	2.37	57	0.0065
Spatial segregation and species discrimination combined	-9.4	2.04	30	<0.001

732 *n* = 25 population pairs

733

734

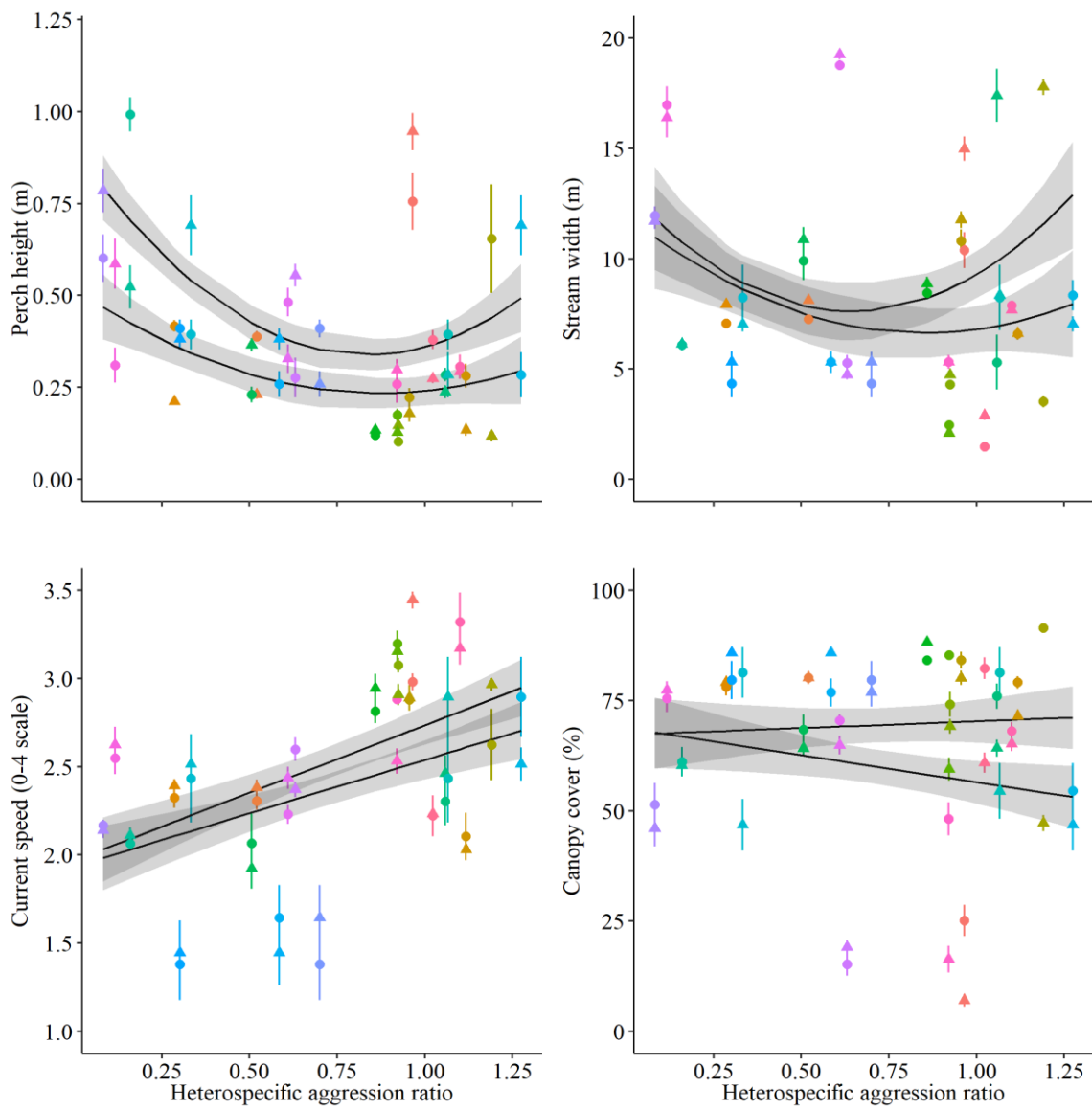
735 **Figure 1**



736

737

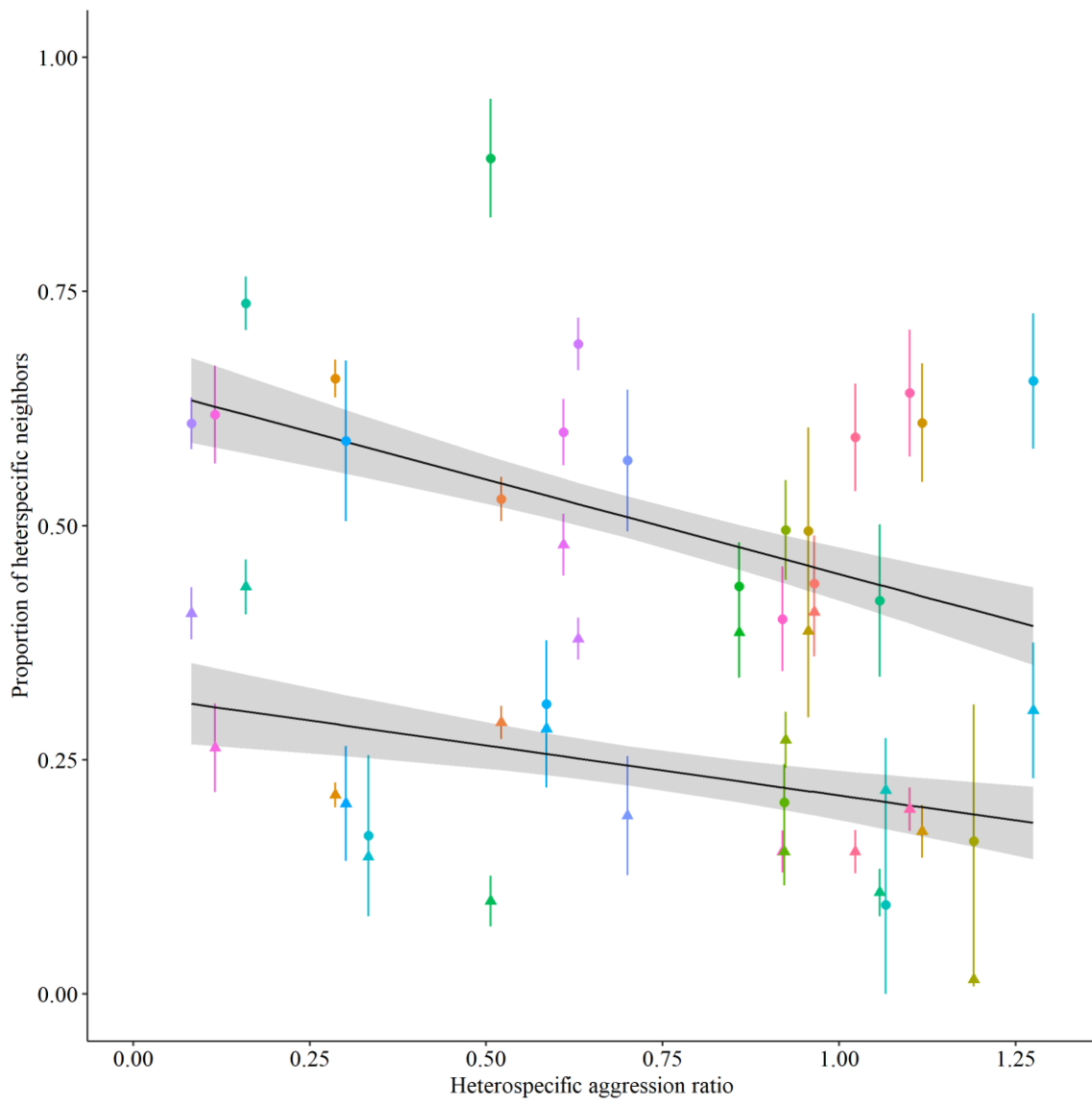
738 **Figure 2**



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740

741 **Figure 3**



742

743