

1 **Title:** Reproductive interference hampers species coexistence despite
2 conspecific sperm precedence

3

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17

18 **Abstract**

19

20 Negative interspecific mating interactions, known as reproductive interference, can hamper
21 species coexistence in a local patch and promote niche partitioning or geographical
22 segregation of closely related species. Conspecific sperm precedence (CSP), which occurs
23 when females that have mated with both conspecific and heterospecific males preferentially
24 use conspecific sperm for fertilization, might contribute to species coexistence by mitigating
25 the costs of interspecific mating and hybridization. We examined whether two closely related
26 species exhibiting CSP can coexist in a local environment in the presence of reproductive
27 interference. First, using a behaviourally explicit mathematical model, we demonstrated that
28 two species characterized by negative mating interactions are unlikely to coexist because the
29 costs of reproductive interference, such as loss of mating opportunity with conspecific
30 partners, are inevitably incurred when individuals of both species are present. Second, we
31 experimentally demonstrated differences in mating activity and preference in two *Harmonia*
32 ladybird species known to exhibit CSP. According to the developed mathematical model of
33 reproductive interference, these behavioural differences should lead to local extinction of *H.*
34 *yedoensis* because of reproductive interference by *H. axyridis*. This prediction is consistent
35 with field observations that *H. axyridis* uses various food sources and habitats whereas *H.*
36 *yedoensis* is confined to a less preferred prey item and a pine tree habitat. Finally, by a
37 comparative approach, we showed that niche partitioning or parapatric distribution, but not
38 sympatric coexistence in the same habitat, is maintained between species with CSP belonging
39 to a wide range of taxa, including vertebrates and invertebrates living in aquatic or terrestrial
40 environments. Taken together, these results lead us to conclude that reproductive interference
41 generally destabilizes local coexistence even in closely related species that exhibit CSP.

42

43 **Key words:** community dynamics, competitive exclusion, host specialization, niche
44 partitioning, reproductive interference, reproductive isolation, resource competition

45

46

47 **Introduction**

48

49 Restrictions to local coexistence among phylogenetically related species are closely related to
50 niche partitioning and the diversification of resource use traits, which help to determine
51 community assemblages at both local and regional scales (Schluter 2000, Grant and Grant
52 2011, Losos 2011). Therefore, understanding the mechanisms that restrict local coexistence is
53 of fundamental importance in ecology and evolution. Negative interspecific mating
54 interaction, that is, reproductive interference, is one mechanism that can drive species
55 exclusion at local scale and subsequent niche partitioning among species (Gröning and
56 Hochkirch 2008). Reproductive interference has been theoretically demonstrated to hamper
57 species coexistence in a homogeneous environment even in ecologically neutral species with
58 similar growth rates and abilities to compete for shared resources (Kuno 1992, Konuma and
59 Chiba 2007, Crowder et al. 2011, Nishida et al. 2015, Kyogoku and Sota 2017). Moreover,
60 empirical studies have also reported that reproductive interference contributes to niche
61 partitioning between congeneric species with overlapping mating signals, including in frogs
62 (Ficetola and Bernardi 2005), birds (Vallin et al. 2012), mites (Takafuji et al. 1997), and
63 insects (butterflies, Friberg et al. 2013; grasshoppers, Hochkirch et al. 2007; ladybirds,
64 Noriyuki et al. 2012). Therefore, reproductive interference is a determinant of local and
65 regional species diversity in a wide range of animal taxa in nature, though its significance in

66 community ecology has been underestimated for decades (Gröning and Hochkirch 2008,
67 Kyogoku 2015).

68 A number of mechanisms, however, are reported to mitigate the negative impacts of
69 reproductive interference on the coexistence of species occupying the same niche, including
70 plastic responses in reproductive traits (Otte and Hilker 2016), continued dispersal to new sets
71 of ephemeral resource patches (Ruokolainen and Hanski 2016), and reinforcement of
72 reproductive isolation (Bargielowski et al. 2013). One possible mitigating mechanism is
73 conspecific sperm precedence (CSP), where females that have mated with both conspecific
74 and heterospecific males preferentially use conspecific sperm for fertilization (Howard 1999).
75 Such females might experience fewer costs associated with interspecific mating and
76 hybridization (i.e., waste of gametes), because most or all of their offspring will be pure
77 conspecifics (Nakano 1985, Veen et al. 2001, Marshall et al. 2002). In addition, in various
78 animals, mating order has been shown to have no influence on whether a female is able to
79 preferentially use conspecific sperm (Howard et al. 1998, Marshall et al. 2002), suggesting
80 that complete CSP can largely eliminate the negative impact of interspecific mating provided
81 that females have mated with at least one conspecific male before the onset of oviposition or
82 birthing (Marshall et al. 2002). CSP has been reported in a variety of animal taxa, including
83 sea urchins (Geyer and Palumbi 2005), mussels (Klibansky and McCartney 2013), crickets
84 (Howard et al. 1998), fruit flies (Price 1997), beetles (Fricke and Arnqvist 2004, Rugman-

85 Jones and Eady 2007), fishes (Yeates et al. 2013), and mice (Dean and Nachman 2009), and
86 thus potentially plays an important role in species coexistence. Although CSP has attracted
87 much attention as a driver of speciation through post-mating and pre-zygotic reproductive
88 isolation (Howard et al. 1998; Howard 1999), it is still unclear whether CSP can sufficiently
89 ameliorate the cost of reproductive interference to promote stable coexistence of closely
90 related species in the same local environment.

91 CSP may not fully function as a barrier against reproductive interference. Under
92 imperfect species discrimination, individual females may incur a variety of costs as a result of
93 interactions with heterospecific males during the reproductive process, such as reduced
94 longevity and oviposition rates (Kawatsu and Kishi 2017), physical damage caused by
95 interspecific copulation (Kyogoku and Sota 2015), and loss of opportunity to mate with
96 conspecific partners (Thum 2007, Noriyuki et al. 2012, Ramiro et al. 2015), as well as the
97 production of unviable hybrid offspring (Todesco et al. 2016). CSP alone might be insufficient
98 to compensate all of these potential costs of reproductive interference. In addition, adaptive
99 behaviours of females and males can prevent multiple matings by females and consequently
100 make the CSP mechanism useless. For example, studies on sexual conflict have shown that
101 females are likely to avoid multiple matings when the benefit is low (Eberhard 1996, Arnqvist
102 and Rowe 2005). Moreover, to prevent sperm competition, males often try to prevent females
103 from mating multiple times, for example, by mate guarding after copulation (Alcock 1994),

104 by placing a physical plug in female reproductive organs (Matsumoto and Suzuki 1992, Polak
105 et al. 2001), or by insertion of a chemical that inhibits remating receptivity (Scott 1986,
106 Gillott 2003, Himuro and Fujisaki 2008). Therefore, to evaluate the ecological role of CSP in
107 species coexistence, various behavioural and physiological mechanisms affecting the
108 reproductive process must be taken into account.

109 In this study, we examined whether CSP can mitigate the effect of reproductive
110 interference in two closely related species so that they are able to coexist in a local
111 environment. We adopted a tripartite approach. First, we developed a behaviourally explicit
112 mathematical model to analyse behavioural and demographic factors affecting local species
113 coexistence, with a focus on the multiple copulation rate, mating preference toward
114 conspecific or heterospecific partners, and the initial population densities of the two species.
115 Second, we conducted mating experiments with two predatory ladybird species, *Harmonia*
116 *axyridis* and *Harmonia yedoensis*, to test the predictions of the mathematical model. CSP has
117 been detected in both these species (Noriyuki et al. 2012), and they occupy different niches in
118 nature; *H. axyridis* is a generalist that feeds on various species of preferred aphids, whereas *H.*
119 *yedoensis* specializes on the giant pine aphid, which is a highly elusive prey item and
120 nutritionally poor for larval development (Noriyuki et al. 2011, Noriyuki and Osawa 2012). In
121 addition, the reproductive success of *H. yedoensis* females is strongly decreased in the
122 presence of *H. axyridis* males, suggesting that *H. yedoensis* might utilize the less preferred

123 food and habitat to avoid reproductive interference from *H. axyridis* (Noriyuki et al. 2012).
124 Third, we investigated the general consequences of CSP on species coexistence in nature by
125 compiling published data on pairs of species in which CSP has been detected and found that
126 such species pairs generally show niche separation (habitat and food source) or
127 geographically separate distributions. We concluded from our results that CSP does not reduce
128 the overall cost of reproductive interference sufficiently to allow the interacting species to
129 coexist in the same local environment.

130

131 **Materials and methods**

132

133 **Mathematical model**

134

135 We modelled a community of two species (X and Y), with density $N_X(t)$ and $N_Y(t)$,
136 respectively, in generation t , inhabiting a single patch. The two species interact through
137 resource competition as well as through reproductive interference, but they are ecologically
138 neutral in terms of the total number of offspring per capita that survive to maturation (denoted
139 r), density-dependent regulation (denoted v), and interspecific competitive strength (denoted
140 b). We assumed a sex ratio of 1:1 (though we found that the ratio does not affect the results;
141 see Kyogoku and Sota 2017), and, for the sake of simplicity, at most two instances of

142 copulation per female. Finally, we assumed that females are not always capable of correctly
143 assessing the species identity of their mating partner; as a result, interspecific mating can
144 occur even after intraspecific mating (as is the case in *H. yedoensis* and *H. axyridis*).

145 Species X and Y can differ with respect to the rate at which females accept males as
146 mates (Fig. 1). Specifically, a virgin X-female (i.e., a female of species X) accepts a mating
147 attempt by an X-male with probability $p_{X|X}$ and a Y-male with probability $p_{X|Y}$, and a once-
148 mated female accepts a mating attempt by an X-male with probability $q_{X|X}$ and with a Y-male
149 with probability $q_{X|Y}$. Similarly, the probabilities of a Y-female accepting a mating attempt by
150 a male in the corresponding situations are $p_{Y|Y}$, $p_{Y|X}$, $q_{Y|Y}$, and $q_{Y|X}$.

151 The frequencies of X-males and Y-males (among all males in the community) are as
152 follows:

153 [Equation 1]

$$154 \quad f_X = \frac{N_X}{N_X + N_Y}, f_Y = \frac{N_Y}{N_X + N_Y}. \quad (1)$$

155 We denote the expected reproductive output by a single X-female or a single Y-female by E_X
156 or E_Y , respectively. Parameter c tunes the intensity of reproductive interference ($0 < c < 1$)
157 and reflects the degree of interspecific overlap in the reproductive niche; thus, the expected
158 reproductive output of an X- or Y-female is calculated as:

159 [Equation 2]

$$160 \quad E_X = \left((1 - c) + c \cdot \frac{(p_{X|X} + p_{X|Y} \cdot q_{X|X} f_Y) f_X}{f_X \cdot p_{X|X} + f_Y \cdot p_{X|Y}} \right) r, \quad (2)$$

$$161 \quad E_Y = \left((1 - c) + c \cdot \frac{(p_{Y|Y} + p_{Y|X} \cdot q_{Y|Y} f_X) f_Y}{f_Y p_{Y|Y} + f_X p_{Y|X}} \right) r,$$

162 (see Appendix A). Within the parentheses on the right side of each Eq. (2), $(1 - c)$ represents
163 reproductive success independent of density and frequency, and the second term represents
164 the product of reproductive interference intensity (c) and the conditional probability that,
165 given a non-virgin, a single female mates with a conspecific male at least once.

166 To model the population dynamics under intra- and interspecific competition, we used
167 the Beverton–Holt model of community dynamics (Beverton and Holt 1957, May and Oster
168 1976, Ackleh and Salceanu 2014). Specifically, we assume that regulation occurs among
169 adults, followed by reproduction. Under this assumption, the dynamics are as follows:

170 [Equation 3]

$$171 \quad N_X(t + 1) = \frac{N_X(t)E_X(t)}{1 + vN_X(t) + vbN_Y(t)}, \quad (3)$$

$$172 \quad N_Y(t + 1) = \frac{N_Y(t)E_Y(t)}{1 + vbN_X(t) + vN_Y(t)}$$

173 where t represents the generation, $r \geq 1$ represents the life-time survival rate (subsuming the
174 total number of eggs per capita), $v \geq 0$ tunes density dependence in regulation, and b ($0 \leq$
175 $b \leq 1$) tunes the strength of interspecific resource competition. Throughout this analysis, we
176 set $v = 1$, which does not cause any loss of generality (Ackleh and Salceanu 2014). By using
177 the underlying link between a continuous-time logistic equation and the discrete-time
178 Beverton–Holt model (May and Oster 1976), we approximate the dynamics by the following
179 ordinary differential equations (ODE):

180 [Equation 4]

$$181 \quad \frac{dN_X(t)}{dt} = \frac{N_X(t)E_X(t)}{1+(N_X(t)+bN_Y(t))} - N_X(t), \quad (4)$$

$$182 \quad \frac{dN_Y(t)}{dt} = \frac{N_Y(t)E_Y(t)}{1+(bN_X(t)+N_Y(t))} - N_Y(t).$$

183 All variables and parameters are defined in Table 1. The community equilibrium is obtained
184 by setting Eqs. (4) to zero. We also carry out a basic local stability analysis of the equilibrium
185 of the dynamical system to determine possible equilibrium states. Specifically, we identified
186 conditions leading to *species exclusion* (i.e., only one species persists) or *coexistence* (i.e.,
187 both species coexist).

188 We also visualized the steady states by a numerical approach, first (i) evaluating the
189 eigenvalues of the Jacobi matrix of equilibria and then (ii) depicting the phase portraits (using
190 Mathematica 11.2.0; Wolfram Research 2017). For the eigenvalue analyses, we first checked
191 the number of feasible equilibria ($N_X, N_Y \geq 0$) given the community dynamics and then
192 numerically evaluated the real part of the eigenvalues associated with the corresponding
193 equilibria.

194

195 **Experiment**

196 We collected adults of two ladybird species from Japanese red pine (*Pinus densiflora* Sieb. et
197 Zucc.) at the University of Tokyo Tanashi Forest (139°32'E, 35°44'N), Tanashi city, Tokyo,
198 during April 2014, and at the Kumagaya campus of Rissho University (139°36'E, 36°10'N)

199 and the Hirose Wild Birds Forest (139°35'E, 36°14'N), Kumagaya city, central Japan, during
200 April 2015. In the laboratory, we maintained females individually in plastic Petri dishes (9 cm
201 in diameter by 1.5 cm high) at 25 °C, and fed them each day with a surplus of frozen *Ephestia*
202 *kuehniella* Zeller eggs (Beneficial Insectary, Ontario, Canada) to ready them for reproduction.
203 In total, 15 *H. yedoensis* and 8 *H. axyridis* females in 2014 and 10 *H. yedoensis* and 9 *H.*
204 *axyridis* females in 2015 produced a sufficient number of egg clutches for our experiments. In
205 addition, in 2015, we collected 32 *H. yedoensis* egg clutches and 41 *H. axyridis* egg clutches
206 that had been oviposited on the leaves and branches of Japanese red pine trees at the Hirose
207 Wild Birds Forest. We fed the hatched offspring from both laboratory-laid and wild-collected
208 egg clutches with a mixture of sucrose, dried yeast, and powdered drone honeybee (following
209 Nijjima et al. 2000) to the adult stage in plastic cases (each 12.5 cm in diameter by 9.5 cm
210 high) containing wood wool as a substrate on which they could walk. We recorded the date of
211 emergence, body length (to the nearest 0.01 mm), and elytra colour (black or red) of all newly
212 emerged adults as possible factors affecting mating preference, and used these virgin
213 individuals for the following behavioural experiments to standardize the mating experience.
214 Because it takes approximately 1 month for most individuals of both *H. yedoensis* and *H.*
215 *axyridis* to mature sexually after they emerge as adults (Okada, Nijjima & Toriumi 1978), we
216 reared the newly emerged adults individually in plastic Petri dishes for at least 30 days,
217 providing them with frozen *E. kuehniella* eggs every other day, before using them in mating

218 experiments. In addition, we excluded egg clutches from the wild-caught mothers that
219 produced only female offspring (two *H. yedoensis* females in 2014 and one *H. axyridis* female
220 in 2015) because they were likely to be infected with male-killing bacteria (Noriyuki et al.
221 2014, 2016), to avoid any confounding effects of male-killing bacteria on the host mating
222 behaviour (Majerus 2003).

223 In the mating experiment, we kept one female (*H. yedoensis* or *H. axyridis*) and one male
224 (*H. yedoensis* or *H. axyridis*) together in a small Petri dish (5 cm in diameter) on a laboratory
225 bench at room temperature (25 °C) under constant fluorescent lighting. We never placed
226 females with sibling males (i.e., individuals produced by the same wild-caught mother or
227 from the same wild-collected clutch) to preclude any effects of inbreeding avoidance on
228 mating behaviour. We observed the occurrence of male mating attempts, female rejection
229 behaviour, and successful copulation in each experimental session (see Noriyuki et al. 2012
230 for the definition of these behaviours). In 2014, we visually observed mating activities during
231 15-min sessions. In 2015, we used videocameras (HC-V480, Panasonic, Osaka, Japan) to
232 record experimental sessions for at least 6 hours (up to 20 hours) and then watched the videos
233 to analyse mating behaviours. In the 2014 experiments, each pair was allowed to mate after
234 the 15-min session until copulation was completed. In the 2015 experiments, multiple
235 copulations were allowed in the same experimental session. Note that Noriyuki et al. (2012)
236 reported that the mean duration of copulation was 228 min in *H. yedoensis* and 124 min in *H.*

237 *axyridis* under similar experimental conditions. In both 2014 and 2015, we reused virgin and
238 non-virgin individuals after the experimental session for other sessions to analyse the effects
239 of mating experience on subsequent mating behaviour.

240 To examine the effect of mating experience in virgins and non-virgins on the copulation
241 rate in each species, we analysed the proportion of experimental sessions that included
242 successful copulation (at least one in the 2015 experiments) by a generalized linear mixed
243 model with a binomial error structure using the `glmer` function of the `lme4` library (Bates et al
244 2015) of the R software package (version 3.4.2, R Core Team 2017). Similarly, we compared
245 the mating rate between intra- and interspecific mating trials in virgin and non-virgin females.
246 Moreover, we analysed mating preferences of both males and females to determine factors
247 responsible for the copulation rate. First, we evaluated male preference by the proportion of
248 experimental sessions that included at least one male mating attempt, whether or not it was
249 followed by successful mating. Second, we examined the female preference by calculating the
250 proportion of male mating attempts that elicited female rejection behaviour. In all analyses,
251 we also incorporated the date of emergence, body length, and elytra colour of females and
252 males as fixed effects, and the identity of the mother of the female and that of the male as a
253 random term. We analysed data from the experiments in 2014 and 2015 separately because of
254 the differences in the source populations and the specific experimental conditions.

255 Furthermore, we applied signal detection theory (Green and Swets 1966) to disentangle

256 the mechanism of decision making in males and females who need to choose conspecific
257 mating partner over heterospecifics. We computed two statistics, d' and β , where d' is signal
258 strength (a higher value indicates that the mating signal from conspecifics is more readily
259 detected), and β reflects an individual's mating strategy. $\beta \approx 1.0$ indicates unbiased decision
260 making; $\beta \approx 0.0$ indicates a bias towards mating with either a conspecific or heterospecific
261 individual (i.e., a liberal strategy); and $\beta > 1.0$ indicates a bias towards rejection of mating
262 with either a conspecific or heterospecific individual (i.e., a conservative strategy). d' and β in
263 response to signals (male mating attempt and female rejection behaviour) in each species were
264 computed by using the `dprime` function of the `neuropsychology` library for the R software
265 package (Makowski 2017). To visualize the decision-making performance in response to both
266 male mating attempts and female rejection behaviour, we calculated the receiver operating
267 characteristic (ROC) curve, which compares the sensitivity (the true positive rate, plotted on
268 the y -axis) with the specificity (the false positive rate, plotted on the x -axis), for the signal
269 detection results by using the `ROCR` package for R (Sing et al. 2005). Essentially, the closer
270 an ROC curve is to the upper left corner, the better the decision-making accuracy, and the
271 closer the curve is to the diagonal line of the panel (i.e., $y = x$), the more likely that the result
272 is owing to chance alone (Carter et al. 2016). In addition, we used the DeLong method in the
273 `pROC` package for R (Robin et al. 2011) to statistically compare the area under the ROC
274 curve (AUC) between species in each experiment year.

275

276 **Comparative study**

277 We performed a literature survey, using the ISI Web of Science

278 (<https://webofknowledge.com/>) on 30 November 2017 and the key phrase “conspecific sperm

279 precedence”, to identify congeneric pairs of animal species in which CSP had been detected in

280 at least one of the pair. In addition, we screened the reference lists of two review papers for

281 CSP (Howard 1999, Marshall et al. 2002) to locate additional pairs. We classified the

282 geographic distributions and niches of each pair into one of four categories: (1) sympatry,

283 geographical distribution of the two species largely overlaps with little if any niche separation

284 in the sympatric area; (2) niche partitioning, geographical distributions of the two species

285 overlap with niche partitioning at local scale (e.g., separation by food, habitat, or seasonality)

286 especially at the reproductive stage; (3) parapatry, geographical distributions of the two

287 species do not overlap but are adjacent with a narrow contact (hybridization) zone; or (4)

288 allopatry, geographical distributions of the two species do not overlap and are not adjacent.

289 We excluded species with cosmopolitan, human-mediated distributions (e.g., *Drosophila*

290 *simulans*, *Tribolium* flour beetles, and *Callosobruchus* bean weevils) from the analysis

291 because their habitats and distributions in the natural environment are unclear. In total, we

292 analysed 24 species pairs of marine invertebrates, terrestrial insects, and vertebrates.

293

294 **Results**

295

296 **Mathematical model**

297 *Equilibria*

298 We found dynamic population equilibria, designated by an asterisk (*), on (i) the N_X -axis (i.e.,
299 $N_X^* > 0, N_Y^* = 0$), (ii) the N_Y -axis (i.e., $N_X^* = 0, N_Y^* > 0$), or (iii) in the interior (i.e., $N_X^* >$
300 $0, N_Y^* > 0$). The boundary equilibria (as a result of competitive exclusion) are given by

$$301 \quad B_X = (r - 1, 0), B_Y = (0, r - 1)$$

302 whereas the interior equilibrium did not have analytical formula (note that because we assume
303 $r > 1$, boundary equilibria were always feasible).

304 *Stability analyses*

305 The stability conditions for the equilibria (species exclusion or coexistence) were determined
306 from the eigenvalues of a Jacobi matrix around the focal equilibrium (more details are given
307 in Appendix B). The necessary condition for a stable equilibrium resulting in extinction of one
308 of the two species is given by:

309 [Equation 5]

$$310 \quad b + c \frac{r}{r-1} > 1. \quad (5)$$

311 In particular, $c = 1$ (i.e., reproductive niches of the species overlap completely) necessarily
312 leads to competitive exclusion given the parameter set for p and q used in our analysis (Fig.

313 2), even in the absence of interspecific resource competition (i.e., $b = 0$). See Appendix C for
314 the numerical procedures for basins of attraction.

315 We note here that, if the two species are highly symmetric in terms of p and q values,
316 then more outcomes become possible; in particular, species exclusion and coexistence states
317 can be stable simultaneously (“bi-stable”), in agreement with Kishi and Nakazawa (2013) and
318 Kyogoku and Sota (2017). Our particular intention here, however, is to explore the effects of
319 asymmetry in mating behaviour (p and q values) on the community dynamics in our
320 experimental system. For more details about the consequences of symmetric p and q values,
321 see Appendix D. Also, it is possible to incorporate differences in the number of mating
322 attempts in a given time period (i.e., mating activity) such that the encounter rate with an X-
323 or Y-male can be biased towards either species relative to their frequency in the community (f_X
324 and f_Y); however, changes in the encounter rate did not change the results dramatically,
325 although species exclusion became more likely (see Appendix D for more information).

326

327 **Experiment**

328 Mating experience did not have a significant effect on the rate of copulation in either the 2014
329 or the 2015 experiment (Fig. SI 4, Table S1); therefore, virgin and non-virgin females were
330 pooled in the following analyses. The copulation rate was higher in *H. axyridis* females than
331 in *H. yedoensis* females, especially in the 2014 experiments, although the difference was not

332 statistically significant (Fig. 3, Table S2). In the 2015 experiment, *H. axyridis* was more likely
333 to mate with conspecifics, whereas no such assortative mating pattern was observed in *H.*
334 *yedoensis*; that is there was a significant interaction effect between female species and species
335 identity of the mating partner (conspecific or heterospecific; Fig. 3, Table S2). In both the
336 2014 and 2015 experiments, *H. axyridis* males more frequently attempted to mate with
337 conspecific females, whereas *H. yedoensis* males did not show a significant preference
338 towards conspecific females (Table S3). *Harmonia axyridis* females were more likely than *H.*
339 *yedoensis* females to refuse mating attempts by conspecific males, especially in the 2014
340 experiment (Table S4); however, both coercive mating and copulation failure occurred in both
341 species following female rejection behaviour.

342 In the signal detection analysis results, d' in response to male mating attempts was higher
343 in *H. axyridis* than in *H. yedoensis* in both 2014 and 2015 (Table S5). Further, the AUC for
344 male mating attempts was significantly higher in *H. axyridis* than in *H. yedoensis* in both
345 2014 and 2015 (Fig. 4, Table 2). By contrast, no consistent pattern in female rejection
346 behaviour was detected between species or years in the signal detection analysis, probably in
347 part because of the small sample size (Table S6). The AUC results for female rejection
348 behaviour was also not significantly different between species in either experiment year (Fig.
349 4, Table 2).

350

351 (c) Comparative study

352 We found spatial separation at both local (niche partitioning) and regional scales (parapatry or
353 allopatry) among species pairs exhibiting CSP, including in marine abalones, freshwater
354 fishes, terrestrial insects, birds, and mice (Table 3). We observed parapatry mainly in
355 Orthoptera (crickets and grasshoppers). We detected sympatry without apparent niche
356 partitioning in 6 of 24 species pairs, especially in aquatic invertebrates such as mussels,
357 starfishes, and sea urchins.

358

359 **Discussion**

360

361 Our results suggest that reproductive interference is likely to hamper stable species
362 coexistence in a local patch even when the interacting species exhibit CSP. Our experimental
363 results for two species in which CSP has been detected showed that the mating rate in a given
364 period was higher in *H. axyridis* females than in *H. yedoensis* females (Fig. 3), and that *H.*
365 *axyridis*, but not *H. yedoensis*, was more likely to copulate with a partner of its own species
366 (Figs. 3 and 4). Our mathematical model indicated that these observed behavioural differences
367 between these *Harmonia* species have a community-level consequence: namely, *H. yedoensis*
368 becomes extinct in a local patch because of reproductive interference from *H. axyridis* (Figs.
369 SI 2 and SI 3). Furthermore, our comparative study of species pairs exhibiting CSP

370 demonstrated that parapatric distribution or niche partitioning, but not sympatric coexistence
371 in the same habitat, can be maintained between two closely related species of a wide range of
372 taxa, including both vertebrates and invertebrates living in either aquatic or terrestrial
373 environments (Table 3). Taken together, these results lead us to conclude that CSP does not
374 generally promote local coexistence between closely related species with overlapping
375 reproductive niches.

376 Our experiment using *Harmonia* ladybirds, combined with our theoretical analysis,
377 clarified the behavioural mechanisms of species exclusion. The rate of copulation was not
378 significantly different between virgin and non-virgin females in the two *Harmonia* species
379 (Fig. SI 4), suggesting that mating experience did not affect the reproductive success of
380 individuals or the subsequent population dynamics in these species. However, the results of
381 our signal detection analysis indicated that *H. axyridis* males easily distinguish and choose
382 conspecific females over heterospecific females (Fig. 4, Table 2), whereas mating rates with
383 conspecifics was low in *H. yedoensis* (Fig. 3). Our mathematical model demonstrated that, in
384 the situations examined by our experiments, *H. axyridis* is likely to mate with a conspecific
385 partner at least once before oviposition begins, whereas *H. yedoensis* females, even though
386 they exhibit CSP, are incapable of producing viable offspring in the presence of *H. axyridis*
387 males, with the result that *H. axyridis* is predicted to exclude *H. yedoensis* from the local
388 patch (Fig. 2). This prediction is consistent with the niche partitioning observed in the field,

389 where *H. axyridis* feeds on preferred prey items on various types of trees and *H. yedoensis*
390 specializes in highly elusive prey on only pine trees. The pine habitat may function as a refuge
391 for *H. yedoensis*, where it can avoid reproductive interference from *H. axyridis* (Noriyuki et
392 al. 2012).

393 Our mathematical model highlighted the behavioural mechanisms that affect the
394 asymmetry of reproductive interference and subsequent species exclusion. Although the
395 classic theory of interspecific competition postulates that species exclusion occurs through
396 exploitative competition for shared resources (Chesson 2000), our model results demonstrated
397 that interference interactions during the reproductive stage hamper the coexistence of two
398 species even when they demonstrate equal competitive strength for resources. In addition, our
399 model results showed that slight differences in mating activity, mating preference, and
400 remating acceptance determine which of two interacting species is superior with respect to
401 reproductive interference (Figs. SI 2 and SI 3), whereas previous theoretical studies on
402 reproductive interference did not fully take into account the consequences of behavioural
403 processes on population dynamics and species' fates (Yoshimura and Clark 1994, Kishi and
404 Nakazawa 2013, Kyogoku and Sota 2017). In addition, we found that species exclusion is
405 more likely to occur for a wide range of initial population densities of the two species when
406 the intensity of reproductive interference is high (Fig. 2). This finding means that closely
407 related species are unlikely to coexist in the same environment if they have similar mating

408 signals or if they share a reproductive niche in space and time; as a result, niche partitioning
409 or geographical segregation of the species is likely to occur.

410 Our comparative study found a separation of niche use or geographical distributions
411 between species pairs with CSP in a range of taxa (Table 3). This finding suggests that CSP
412 alone does not allow these species pairs to coexist in the same local environment. The pattern
413 corresponds found in our comparative study is consistent with the prediction of our
414 mathematical model that two interacting species are unlikely to coexist when c (intensity of
415 reproductive interference) is high (Fig. 2). However, sympatric coexistence without apparent
416 niche separation was also detected, especially in free-spawning marine invertebrates such as
417 mussels, starfishes, and sea urchins (Table 3). There are several possible reasons that can
418 account for the discrepancy between our model prediction and the actual pattern in nature in
419 these cases. First, niche separation might actually exist, but, perhaps because of limited field
420 survey data, it may not have been recognized. In fact, fine-scale differences in adult habitat
421 and the timing of spawning have been detected in closely related marine invertebrate species
422 (Lindberg 1992, Fogarty 2012). Therefore, it is possible that niche separation has actually
423 occurred to mitigate the cost of reproductive interference in such species. Second, dispersal to
424 new patches can allow overlapping niche use at a local scale even when two species engage in
425 competitive interactions. Especially in marine sessile invertebrates that have high dispersal
426 ability in the larval stage and a sedentary life style in the adult stage, source–sink dynamics

427 (Mouquet and Loreau 2003) and stochastic processes (Paine and Levin 1981) likely promote
428 local species coexistence. Third, in sessile animals, decision making at the pre-mating stage
429 may not be important; females may be likely to accept sperm from conspecific as well as from
430 heterospecific males, which means that CSP makes it possible for them to produce viable
431 offspring. In this situation, therefore, CSP can indeed mitigate the cost of interspecific mating
432 and thus promote species coexistence in the same niche. Clearly, it is important to incorporate
433 life-history characteristics when considering the community-level consequences of
434 behavioural decision making in animals.

435 By including plants, it would be possible to extend our model to more general scenarios
436 of interacting species under imperfect species recognition. Reproductive interference occurs
437 in flowering plants when the stigma receive heterospecific as well as conspecific pollen
438 grains, for example when flowering phenology and pollinators overlap (Matsumoto et al.
439 2010, Runquist and Stanton 2013, Takakura 2013, Nishida et al. 2014). In some cases,
440 however, conspecific pollen tubes preferentially grow and fertilize the ovules (Baldwin and
441 Husband 2010, reviewed in Howard 1999). This phenomenon is called conspecific pollen
442 precedence, and is considered a mechanism of reproductive isolation that prevents
443 hybridization, and consequently, speciation in plants (Howard 1999). Therefore, it is
444 suggested that conspecific pollen precedence in plants, similar to CSP in animals, can mitigate
445 the cost of reproductive interference and lead to species coexistence in the same habitat.

446 Alternatively, as our model predicted, conspecific pollen precedence may be insufficient to
447 allow interacting species to coexist in the same local environment. In fact, in three species of
448 *Iris*, conspecific pollen precedence has been detected together with habitat differences
449 (Carney et al. 1996; Emms et al. 1996), suggesting that reproductive interference destabilizes
450 local coexistence of these species. In future, it would be interesting to examine whether our
451 model is applicable to plant species by investigating reproductive success in species pairs
452 exhibiting conspecific pollen precedence.

453 In conclusion, our study clarified the ecological significance of CSP by identifying
454 conditions that lead to local species exclusion despite the presence of CSP. This finding is in
455 contrast to those of previous studies of CSP, which have focused on its evolutionary
456 significance, that is, speciation through post-mating pre-zygotic reproductive isolation.
457 Moreover, many CSP studies have not quantified pre-mating behaviours that can affect the
458 reproductive success of females but have instead examined the functioning of CSP by
459 focusing on post-mating, pre-zygotic mechanisms. Importantly, however, it has been
460 documented that the overall costs of reproductive interference, including loss of mating
461 opportunity and decreases in the oviposition rate due to male interference, can lead to the
462 extinction of one of the interacting species even if interspecific mating and insemination does
463 not occur (Kishi et al. 2009, Friberg et al. 2013, Carrasquilla and Lounibos 2015). Therefore,
464 to understand individual reproductive success and community structure of closely related

465 species, pre-mating behaviours should not be neglected.

466

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473

474 **Declarations**

475 The authors declare no competing interest.

476 **References**

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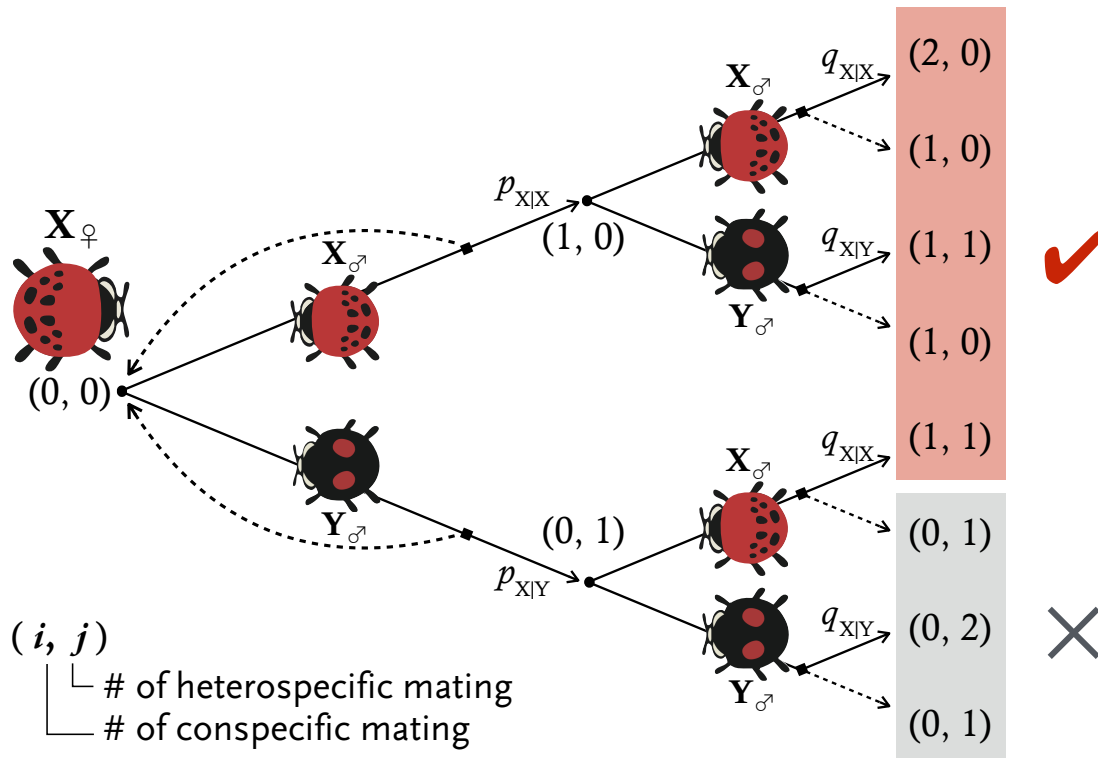
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837 **Figures**

838

Unmated → 1st mating → 2nd mating → Reproduction



839

840

841 **Fig. 1.** Schematic mating decision-making tree for a female of species X according to our

842 mathematical model. Here, (i, j) means a female with i intraspecific matings and j

843 interspecific matings ($1 \leq i + j \leq 2$). A virgin female has state (0, 0), and she accepts a

844 given X-male or Y-male with a probability $p_{X|X}$ and $p_{X|Y}$, respectively. Subsequently, the non-

845 virgin female with state (0, 1) or (1, 0) accepts an X-male or Y-male with probability $q_{X|X}$ or

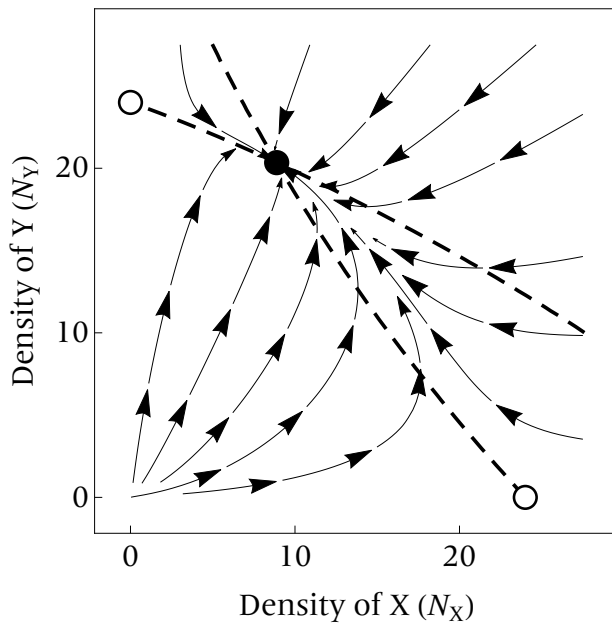
846 $q_{X|Y}$, respectively. The corresponding mating decision-making tree for a Y-female can be

847 obtained by exchanging X and Y. The female states after the second mating that include at

848 least one intraspecific mating ($i \geq 1$) are shaded red; in this case, the female can produce
849 offspring of her own species through CSP. The states of females that failed to copulate with a
850 conspecific male before producing offspring ($i = 0$) are shaded grey.
851

852 **a**

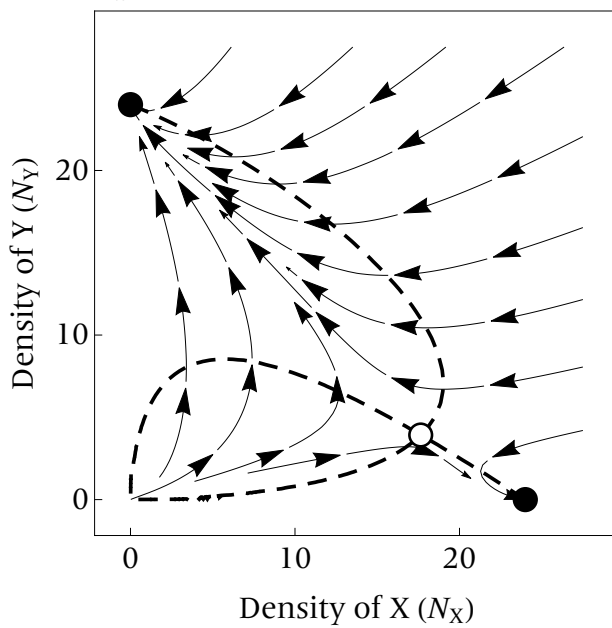
Intensity of RI: 0.5



853

854 **b**

Intensity of RI: 1.



855

856 **Fig. 2.** Phase portraits of the population dynamics according to the original, time-discrete

857 dynamics and the approximated, time-continuous dynamics (i.e. ODE), for varying intensities

858 of reproductive interference (RI; tuned by c). (a) Co-existence is possible when RI intensity is

859 weak ($c = 0.5$). (b) Competitive exclusion occurs when RI intensity is very strong ($c = 1$).

860 Dotted curves, isoclines; arrows: approximated vector fields based on the ODE; open circles,

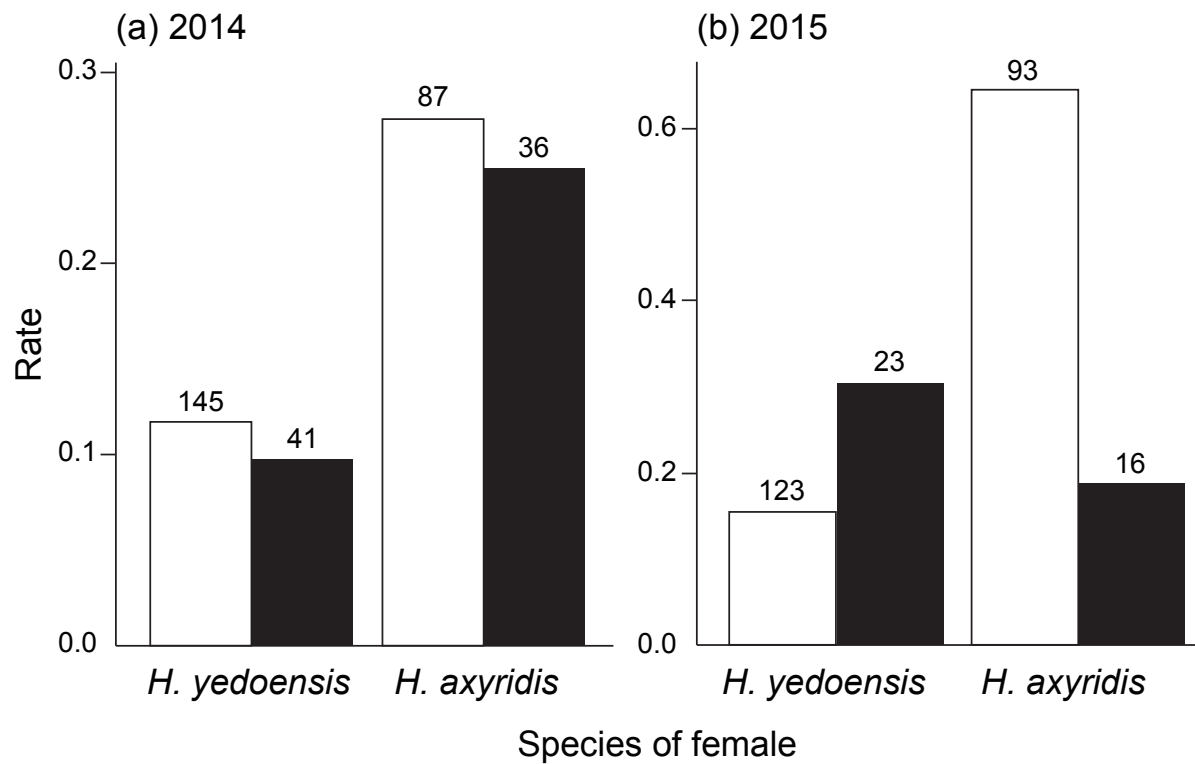
861 unstable equilibria; and closed circles, stable equilibria. The procedure used to produce the

862 figures is described in Appendix C. Probability parameter values: $p_{XX} = 0.4$, $q_{XX} =$

863 0.4 , $p_{XY} = 0.8$, $p_{YY} = 0.8$, $p_{YX} = 0.4$, $q_{YY} = 0.8$; other parameters, default values (see

864 Table 1).

865



866

867

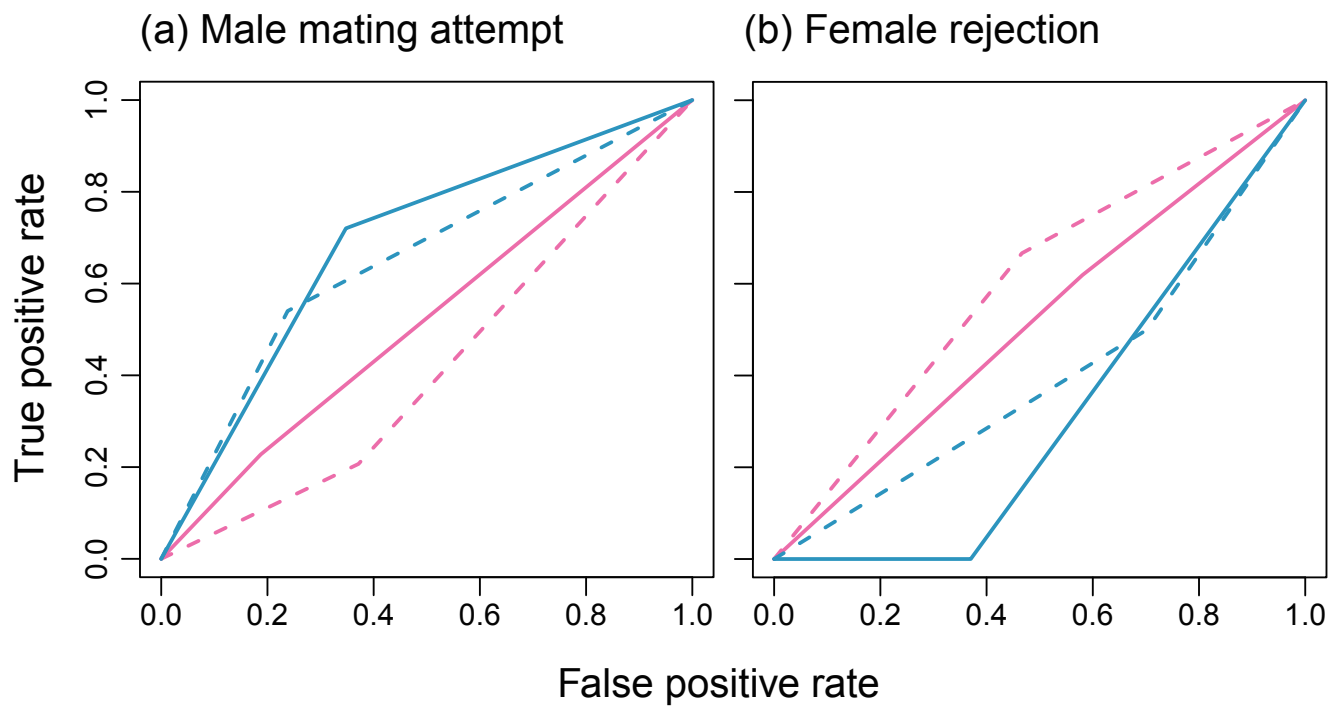
868 **Fig. 3.** Mating rates with conspecific (white) and heterospecific (black) males in the (a) 2014

869 and (b) 2015 experiments. The number of individuals in each category is shown above each

870 bar.

871

872



873

874

875 **Fig. 4.** Receiver operating characteristic curves for (a) mating attempts by males and (b)

876 rejection behaviour in females. In each panel, red and blue lines indicate *H. yedoensis* and *H.*

877 *axyridis*, respectively, and dashed and solid lines indicate the 2014 and 2015 experiments,

878 respectively.

879

880

881 **Table 1.** Parameters included in the model.

882

Parameter/Variable	Definition	Default value (if any)
X or Y	Species label	–
N_X, N_Y	Density of X or Y	Dynamic variable
t	Generation ($t \geq 0$)	–
r	Egg production (per capita)	25
v	Density dependence of resource competition ($v > 0$)	1
b	Strength of interspecific competition ($b > 0$)	0.3
c	Strength of reproductive interference ($0 < c < 1$); probability that a female is subject to a possible heterospecific mating	Varied
$p_{i j}$	Probability that a virgin female of species i accepts a mating attempt by a male of species j , where i and j can be either X or Y	See Fig. 2
$q_{i j}$	Probability that a non-virgin female of species i accepts a mating attempt by a male of species j , where i and j can be either X or Y	See Fig. 2
$f_X (= N_X / (N_X + N_Y))$	Frequency of species X in the population	Dynamic variable
E_X, E_Y	Expected reproductive output (per capita), calculated based upon the mating decision-making tree (see Fig. 1), for species X or Y	–

883

884

885 **Table 2.** Comparison of the area under curve (AUC) values between species. Statistically

886 significant results are shown in boldface.

887

Behaviour	Year	AUC		Statistic		
		<i>H. yedoensis</i>	<i>H. axyridis</i>	<i>D</i>	df	<i>P</i>
Male mating attempt	2014	0.429	0.635	-3.738	298.130	< 0.001
	2015	0.512	0.630	-2.247	220.750	0.026
Female rejection	2014	0.571	0.421	1.648	87.723	0.103
	2015	0.519	0.485	0.481	52.513	0.632

888

1 **Table 3.** Summary of comparative study results.

2

Group	Common name	Species pair	Category	Description	Reference	
Marine invertebrate	Abalone	<i>Haliotis corrugata</i> and <i>H. rufescens</i>	Sympatry	Niche overlap in terms of water depth and habitat	1–3	
		<i>Haliotis cracherodii</i> and <i>H. rufescens</i>	Niche partitioning	Intertidal zone versus kelp forest habitat	3, 4	
		<i>Haliotis fulgens</i> and <i>H. rufescens</i>	Niche partitioning	Shallow versus deep water habitats	2, 3, 5	
	Blue mussel	<i>Mytilus trossulus</i> and <i>M. edulis</i>	Sympatry	Hybrid zone is not narrow	6, 7	
	Starfish	<i>Asterias forbesi</i> and <i>A. rubens</i>	Sympatry	Similar habitats, food resources, and spawning time	8, 9	
	Coral	<i>Montastraea annularis</i> and <i>M. franksi</i>	Niche partitioning	Separation in (slightly overlapped) spawning time	10	
	Sea urchin	<i>Echinometra mathaei</i> and <i>E. oblonga</i>	Sympatry	Slight ecological differences	11	
		<i>Echinometra oblonga</i> and <i>E. sp. C</i>	Sympatry	Slight difference in habitat but similar spawning time	12	
	Terrestrial invertebrate	Cricket	<i>Allonemobius fasciatus</i> and <i>A. socius</i>	Parapatry		13, 14
			<i>Gryllus firmus</i> and <i>G. pennsylvanicus</i>	Parapatry		15, 16
<i>Gryllus bimaculatus</i> and <i>G. campestris</i>			Parapatry		17, 18	
Grasshopper		<i>Chorthippus p. parallelus</i> and <i>C. p. erythropus</i>	Parapatry		19, 20	
		<i>Podisma pedestris</i> races	Parapatry		21, 22	
Ladybird		<i>Epilachna pustulosa</i> and <i>E. vigintioctomaculata</i>	Niche partitioning	Host plant separation	23, 24	
		<i>Harmonia yedoensis</i> and <i>H. axyridis</i>	Niche partitioning	Difference in prey item and habitat	25	
Fruit fly		<i>Drosophila yakuba</i> and <i>D. santomea</i>	Parapatry	Lowland versus highland distributions	26, 27	
Stalk-eyed fly		<i>Teleopsis dalmanni</i> diverged populations	Allopatry		28, 29	
Damselfly		<i>Ischnura graellsii</i> and <i>I. elegans</i>	Niche partitioning	The two species are rarely found in the same localities	30–33	
Vertebrate	Darter fish	<i>Etheostoma barrenense</i> and <i>E. zonale</i>	Sympatry	Not closely related within the genus	34	
		<i>Etheostoma hopkinsi</i> and <i>E. luteovinctum</i>	Allopatry		35	

Salmonid	<i>Salmo salar</i> and <i>S. trutta</i>	Niche partitioning	Spatial and temporal segregation in spawning activities	36–38
Sunfish	<i>Lepomis macrochirus</i> and <i>L. gibbosus</i>	Niche partitioning	Differences in nesting and breeding habits	39, 40
Bird	<i>Ficedula hypoleuca</i> and <i>F. albicollis</i>	Niche partitioning	Separation in breeding habitat	41–43
Mouse	<i>Mus domesticus</i> and <i>M. musculus</i>	Parapatry		44, 45

3

4 1: Vacquier et al. (1990); 2: Cox (1962); 3: Lindberg (1992); 4: Vacquier and Lee (1993); 5: Kresge et al. (2000); 6: Klibansky and McCartney
5 (2014); 7: Gaitán-Espitia et al. (2016); 8: Harper and Hart (2005); 9: Menge (1979); 10: Fogarty et al. (2012); 11: Metz et al. (1994); 12: Geyer
6 and Palumbi (2005); 13: Howard et al. (1998); 14: Howard and Waring (1991); 15: Larson et al. (2012); 16: Harrison and Arnold (1982); 17:
7 Tyler et al. (2013); 18: Veen et al. (2013); 19: Butlin (1998); 20: Butlin and Hewitt (1985); 21: Hewitt et al. (1989); 22: Hewitt (1975); 23:
8 Nakano (1985); 24: Matsubayashi and Katakura (2009); 25: Noriyuki et al. (2012); 26: Chang (2004); 27: Lachaise et al. (2000); 28: Rose et al.
9 (2014); 29: Christianson et al. (2005); 30: Sanchez-Guillen et al. (2011a); 31: Sánchez-Guillén et al. (2011b); 32: Sánchez-Guillén et al. (2013a);
10 33: Sánchez-Guillén et al. (2013b); 34: Williams and Mendelson (2014); 35: Mendelson et al. (2007); 36: Yeates et al. (2013); 37: Heggberget et
11 al. (1988); 38: Jonsson and Jonsson (2009); 39: Immler et al. (2011); 40: Osenberg et al. (1992); 41: Veen et al. (2001); 42: Qvarnström et al.
12 (2009); 43: Vallin et al. (2012); 44: Dean and Nachman (2009); 45: Payseur et al. (2004).

