

1 Sexual conflict promotes species coexistence through negative 2 frequency dependence

3 Miguel Gomez-Llano¹, Sofie Nilén², Iain Moodie³ and Erik I. Svensson²

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5 1. Department of Biological Sciences, University of Arkansas, Fayetteville AR 72701, USA

6 2. Evolutionary Ecology Unit, Department of Biology, Lund University, 223 62, Sweden

7 3. Faculty of Natural Sciences, University of Stirling, FK9 4LA, UK

8 9 **Abstract**

10 A major challenge in community ecology is to understand the mechanisms promoting stable
11 local coexistence. A necessary feature of local coexistence is that species show negative
12 frequency dependence, rescuing rare species from exclusion. However, most studies have
13 focused on ecological differences driving negative frequency dependence, ignoring non-
14 ecological mechanisms such as reproductive interactions. Here, we combined field studies
15 with behavioural and mesocosm experiments to investigate how reproductive interactions
16 within and between species promote coexistence. Our results indicate that the intensity of
17 male mating harassment and sexual conflict increases as species become more common,
18 reducing female productivity and leading to negative frequency dependence. Moreover, field
19 surveys reveal that negative frequency dependence operates in natural settings, consistent
20 with our experimental results. These results suggest that sexual conflict can promote local
21 coexistence and highlights the importance of studying reproductive interactions together with
22 ecological differences to better understand the mechanisms promoting species coexistence.

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24

25 **Significance statement:** Research on the mechanisms promoting local species coexistence
26 have focused on canonical ecological differences that increase intraspecific over interspecific
27 competition. However, one intrinsic factor of species that can promote coexistence are the
28 reproductive interactions. We performed a series of behavioural and mesocosm experiments
29 manipulating species frequencies together with field observations and show that sexual
30 conflict can decrease female fitness when species are common and promote local
31 coexistence. Our results suggest that reproductive interactions are an understudied
32 mechanism that can promote species coexistence even when species are ecologically
33 equivalent.

34

35 **Introduction**

36 Understanding the causes underlying species diversity in ecological communities is a major
37 challenge in both ecology and evolution. Coexistence theory predicts that negative frequency
38 dependence is necessary for local species coexistence (1). If species have a fitness advantage
39 when rare, they can increase from low abundance in a community and hence be rescued from
40 competitive exclusion (1–3). Previous research has focused on how ecological differences
41 between species can cause negative frequency dependence through rare species advantage
42 (4–11), for example through predator susceptibility (8, 9), resource competition (5, 12) and
43 phenology (11, 13). However, many communities are formed by species with little or no
44 ecological differentiation (14–18). How or do such ecologically equivalent species coexist in
45 a community? One possible answer to this question lays on an intrinsic characteristic of many
46 species that can limit species population growth rate and promote species coexistence:
47 reproductive interactions (19–22). Given how widespread sexual reproduction is in the tree of
48 life, it is surprising how understudied reproductive interactions are as a mechanism for
49 species coexistence.

50

51 Reproductive interactions can be categorized into four different groups: intraspecific
52 interactions between the sexes, interspecific interactions between the sexes, intra- and
53 interspecific interactions within the sexes. Importantly, not all reproductive interactions can
54 promote local species coexistence. Intraspecific reproductive interactions between the sexes,
55 such as male mating harassment and the resulting sexual conflict, can reduce female fitness
56 and decrease population growth rate (23–26). Because male mating harassment and its fitness
57 consequences for females is expected to be more intense when a species is common (23, 27–
58 30), sexual conflict can in theory, promote local species coexistence (19, 20, 31, 32).
59 Interspecific reproductive interactions between the sexes, when males mate or attempt to
60 mate with females of another species (e. g., reproductive interference) (33) can lead to
61 positive frequency dependence if females of the rare species suffer more from mating
62 attempts by males of the common species, preventing local coexistence (34–36). Conversely,
63 intraspecific competition within sexes (e. g., conspecific male-male competition) is expected
64 to increase when a species is common (37). Moreover, male-male competition can affect
65 male fitness by reducing longevity and/or male mating success (30, 38), leading to negative
66 frequency dependence and local species coexistence (37, 39, 40). Finally, interspecific
67 reproductive interactions within sexes (e.g., heterospecific male-male competition for mates)
68 (41–43) can reduce male mating success and longevity (30, 37). Because the rare species will
69 suffer more from heterospecific competition, such competition is expected to cause positive
70 frequency dependence and prevent local species coexistence. Although male fitness is not
71 always correlated with population growth, if males have reduced access to females, this could
72 reduce the proportion of fertilized females and decrease population growth through
73 reproductive collapse (44). Therefore, intra- and interspecific reproductive interactions within
74 and between sexes can promote or prevent local species coexistence.

75

76 Importantly, these different types of reproductive interactions are likely to simultaneously
77 operate within a given community. For example, males may compete both with conspecific
78 and heterospecific males for mating territories (37). Because the frequency of these different
79 reproductive interactions are likely to differ in importance and magnitude in different
80 communities, studying only a subset of these interactions will only reveal a partial picture of
81 community dynamics. For example, a previous study on *Calopteryx* damselflies suggested
82 that in the presence of heterospecific male-male competition, the pressure from conspecific
83 male mating harassment decreased and hence the intensity of sexual conflict (30). However,
84 to the best of our knowledge no study has investigated all the different ways by which
85 reproductive interactions within and between species can promote or prevent species
86 coexistence.

87

88 Damselflies (Odonata: Zygoptera) have been widely used in research on sexual selection and
89 sexual conflict, as they have intense reproductive interactions, such as conspecific male-male
90 competition (37, 38, 45), heterospecific male-male competition (30, 37, 42, 43, 46), sexual
91 conflict (25, 28, 30) and heterospecific matings and mating attempts (43, 47, 48). Moreover,
92 they have been intensively used to investigate species coexistence in both the larvae (9, 10,
93 49–54) and less extensively adult stage (37). Therefore, these characteristics of damselflies
94 make them ideal study systems to investigate the role of reproductive interactions in
95 promoting local species coexistence.

96

97 Here, we used a combination of mating experiments, mesocosm experiments across the entire
98 life cycle and surveys of natural damselfly assemblages to investigate if reproductive
99 interactions can promote or prevent local species coexistence. Our focal study organisms are

100 two species of pond damselflies, *Enallagma cyathigerum* and *Ischnura elegans* which are
101 phenotypically very similar and they frequently co-occur (Fig. 1). Specifically we
102 investigated 1) if any of the four types of reproductive interactions (intra- and interspecific
103 within and between sexes) showed signs of frequency dependence; 2), if such reproductive
104 interactions have a fitness cost; 3) if these two species are stably coexisting or only co-
105 occurring (i.e., do they show negative frequency dependence); and 4) if these reproductive
106 interactions and their fitness costs are likely to explain species frequencies and abundance
107 dynamics across generations in natural communities. To answer these questions, we first
108 carried out mating experiments where we manipulated species frequencies and test if the
109 intensity of reproductive interactions changed when species are common compared to when
110 rare. We proceed by quantifying the potential fitness costs of such reproductive interactions
111 by measuring female survival and female productivity in a large multi-generational
112 mesocosm with experimentally manipulated species frequencies. Finally, we quantified
113 community dynamics through density- and frequency-changes at 18 communities across two
114 generations. Taken together, our integrative study investigates if these species are stably
115 coexisting, identifies sexual conflict as a mechanism that can promote local species
116 coexistence, and shows that sexual conflict can explain species frequency changes across
117 generations in natural settings. Our study therefore shows empirical evidence of a non-
118 ecological mechanism promoting species coexistence, highlighting the need of broadening
119 the views from traditional ecological perspectives and further integration of community
120 ecology with evolutionary biology.

121

122 **Methods**

123 *Study species*

124 The common bluetail (*I. elegans*; Fig. 1A) and the common bluet (*E. cyathigerum*; Fig. 1B)
125 are two ecologically similar and closely related damselfly species that are distributed
126 throughout Eurasia with their northernmost range limits in Scandinavia, were they are
127 commonly found in large numbers in ponds and lakes (55). These two damselfly species
128 shared a most recent common ancestor at least 12.6 million years ago (56) and overlap
129 extensively in their adult season (Fig. 1C) and are frequently locally sympatric (Fig. 1D). In
130 southern Sweden where this study took place these species are univoltine (55), and due to
131 their limited dispersal ability (less than 1Km) (57, 58), they can be found in a mosaic of
132 largely discrete populations with different environmental conditions.

133

134 The reproductive behaviours of *Ischnura* and *Enallagma* are very similar. First, males are
135 non-territorial and chase females (often several males at the time) and compete to grab the
136 females by the prothorax using the claspers situated in the tip of their abdomens. If a male is
137 able to find and subsequently clasp a female they form a tandem, after which the female can
138 respond by bending the abdomen to reach the male genitalia and copulate (55) (Fig 1A).
139 Before insemination the males remove the sperm from previous copulations (59, 60).
140 Therefore, females gain few or no benefits from multiple matings, but will experience fitness
141 costs that increase with the number of claspings (28). After copulating females oviposit in
142 emergent vegetation and the larvae grow and overwinter in the aquatic stage (55). Males and
143 females of both species are generalist predators and forage for flying insects near the water
144 (55). During the reproductive season males interact frequently with both con- and
145 heterospecific males, which can reduce male mating success (30, 37, 38, 42, 45, 48). In
146 southern Sweden, where this study took place, adults of both species are found from late
147 spring to late summer (late May to August) to reproduce (Fig 1 C).

148

149 *Are reproductive interactions frequency dependent?*

150 To investigate if any of the four types of reproductive interactions shows frequency
151 dependence, we carried out a mating trial experiment where we varied these two species
152 relative frequencies: common (75%) and rare (25%). We used adult males and females (aged
153 by the stiffness of the wings) from natural populations with no visible signs of external
154 physical harm such as wing damage. We separated the captured individuals by sex and kept
155 them at a density of 10 individuals in netted containers (10.2 cm diameter and 22.9 cm
156 height) during transportation to Stensoffa Ecological Field Station, southern Sweden, where
157 the experiments took place. At the field station we set up males and females in larger netted
158 cages (45 cm diameter and 50 cm height). We added twigs and grasses to each cage to mimic
159 natural vegetation and allow individuals to perch or rest, and a plastic cup with water to
160 prevent desiccation. In each cage we put six individuals of one species (three males and three
161 females) and two individuals of the other species (one male and one female). Thus, in these
162 cages, we had two frequency treatments, both with equal sex ratios: “common” (75%) for the
163 most abundant species and “rare” (25%) for the less abundant species (Supplementary Table
164 1A).

165

166 We marked all males in each cage with individual fluorescent colour powder in the genital
167 area at the base of the abdomen and on the claspers (Fig 1B). After 24 hours, we terminated
168 the experiment and searched for traces of colour dust on the genitalia and prothorax of the
169 females. This technique allowed us to identify how many and which type of males (i.e., con-
170 or heterospecifics) attempted to mate (i.e., clasped) or mated a given female, as these marked
171 males left traces of colour dust in the female prothorax (i.e., mating attempt or clasping) and
172 genitalia (i.e., successful mating) visible under UV-light. This method has previously been
173 successfully used to quantify the degree of short-term male mating harassment (number of

174 male clasplings) and female mating rates in *I. elegans*, *E. cyathigerum* and other damselfly
175 genera (28, 30, 37, 38).

176

177 To estimate the intensity of intraspecific reproductive interactions between sexes, and hence
178 the potential for sexual conflict, we counted the number of male mating attempts (i.e.,
179 number of clasplings per female) on conspecific females in 24 hours. We underscore that this
180 rate of clasplings does not take into account mating attempts that did not end up in clasplings
181 (i.e., chasing of females) or repeated clasplings of females by the same male. Therefore, our
182 measure of sexual conflict is conservative and will underestimate the total costs of male
183 mating harassment to female fitness. To estimate interspecific reproductive interactions
184 between sexes we counted the number of male mating attempts of heterospecific females,
185 using the same procedure (i.e., remnants of coloured dust on the female prothorax or
186 genitalia) in 24 hours. This measure is also a conservative measure of male mating
187 harassment, as it does not take into account heterospecific mating attempts that did not end
188 up in clasplings. Finally, we quantified the costs of intra- and interspecific interactions within
189 sexes as male mating success (mated = 1; not mated = 0). Because male-male competition
190 can reduce male mating success (38), and if conspecific competition is stronger than
191 heterospecific competition, male mating success is expected to decrease when species are
192 common (i. e., negative frequency dependence). Conversely, if heterospecific male-male
193 competition is stronger, male mating success is expected to decrease when species are rare (i.
194 e., positive frequency dependence).

195

196 *Are reproductive interactions costly and do they cause negative frequency dependence and*
197 *rare species fitness advantage?*

198

199 We performed a series of mesocosm experiments under semi-natural conditions in eight large
200 square outdoor cages (3m per side; total volume 27 m³) at the field station (Fig 1E). The aim
201 of this mesocosm experiment was to quantify adult female longevity and per capita female
202 productivity (i. e., the number of emerging female offspring in the next generation per female
203 in the previous generation, a measure that should closely reflect population mean fitness or
204 mean per capita growth rate under different species frequency treatments (common, 75% and
205 rare, 25%). Each cage contained a large water container (600L) with natural vegetation to
206 resemble natural conditions and facilitate oviposition (Fig 1F). Each water container was
207 inoculated repeatedly in the spring preceding these experiments with zooplankton (mainly
208 copepods and *Daphnia*) obtained from nearby ponds and macrophytes obtained from an
209 aquarium shop. This ensured that the damselfly larvae in our experiments would have enough
210 food to forage and grow. A few weeks after inoculations we confirmed by visual inspections
211 that these water containers had reproducing populations of zooplankton in the water. We
212 added six coffee filter papers and small pieces of floating vegetation (*Phragmites australis*)
213 to provide a resting substrate and to facilitate oviposition in these water containers. The
214 outdoor cages were covered with mesh small enough to keep damselflies in and predators
215 out, but wide enough to let smaller insects necessary as food for the foraging adults to enter
216 (25, 30, 37). Importantly, these cages had no predators as we aimed to investigate if intra-
217 and interspecific interactions could cause negative frequency dependence and potentially
218 promote species coexistence. We have showed in previous studies that adult damselfly
219 survival is not affected by total adult density, indicating that prey availability is not an issue
220 in this experimental set up (30).

221

222 In each of these eight outdoor cages we manipulated species frequencies in two treatments
223 with the same frequencies as in the mating trials described above: common (75%) and rare

224 (25%). In each cage, we included 18 conspecifics (six females and 12 males) and six
225 heterospecifics (two females and four males) for a total of 24 individuals per cage. We
226 carried out a total of nine replicates (five for treatment with *I. elegans* being common and
227 four for the treatment with *E. cyathigerum* being common) during the reproductive season
228 (June and July) (Supplementary Table 2A). We also carried out two additional control
229 treatments that would allow us to assess if there could be contamination in our water tanks
230 from damselfly eggs attached to the vegetation or accidentally introduced through the
231 zooplankton inoculation. The control cages contained 24 individuals (eight females and 16
232 males) of one species: two cages with only *I. elegans* (100 %) and two cages with only *E.*
233 *cyathigerum* (100 %). Thus, any *E. cyathigerum* individuals that emerged in the *I. elegans*
234 only control, and *vice versa* were considered as evidence for contamination from the outside.
235 In each mesocosm cage, we marked every individual male and female with a unique number
236 in two of the wings using permanent marker (such marking does not affect flight
237 performance). This made possible measuring individual longevity by visiting these cages
238 every day. We commonly observed marked females that mated and oviposited in the water
239 containers inside the cages during the summer of 2018. In the two subsequent years (2019
240 and 2020), the cages were checked daily during the reproductive season to collect all
241 emerging individuals, which were subsequently sexed and identified to species. Per capita
242 female productivity per species was quantified as the number of female offspring emerged
243 divided by the total number of adult females in the initial generation. Female productivity
244 should be closely connected to female fitness and population growth rate (61).

245

246 *Do these species show negative frequency dependence in nature?*

247 To test if our experiments show patterns consistent with assemblage dynamics (changes in
248 species abundance) in natural populations, we surveyed 18 localities with natural *Ischnura*-

249 *Enallagma* assemblages in southern Sweden during the reproductive season of 2018 and
250 2019, corresponding of two generations (Supplementary Table 3). These sites varied in
251 relative species frequencies (Fig 1D). To quantify species frequencies and densities we
252 visited each site between three and five times per season (May-July) during warm ($>15^{\circ}\text{C}$)
253 days with no rain or strong wind, the most favourable conditions for these damselflies
254 (Supplementary Table 3). During these visits, we captured as many individuals as possible
255 with hand nets for 30 minutes, after which each individual was sexed and identified to
256 species. The relative frequency of each species was taken as the number of individuals of that
257 species divided by the total number of individuals of both species in each season. Species
258 densities were calculated as encountering rate, number of individuals caught per sampling
259 time (i. e., individuals caught per person-minute). Encountering rate has been used previously
260 as a proxy of species density in adult damselflies (28). We calculated the changes in species
261 densities across years (generations) by dividing the density of each species at a given site in
262 2019 to the initial density of the same species in 2018 (number of adults in 2019 per adult in
263 2018).

264

265 *Statistical analysis*

266 Statistical analyses were carried out using generalized linear models assuming poisson
267 (number of con- and heterospecific claspings), binomial (male mating success), negative
268 binomial (longevity) and normal (female productivity) distributions of the residuals. The
269 number of con- and heterospecific claspings, male mating success, female longevity and
270 productivity were all treated as dependent variables. Species frequency, species and their
271 interaction were included as fixed factors. In the analysis of number of con- and
272 heterospecific claspings and male mating success in the mating experiments, we controlled
273 for replicate cage number including it as a covariate. In the model of adult female longevity

274 in the mesocosm experiments, we included the interaction between cage and replicate as
275 random factors. Species density changes in the 18 natural communities was analysed using a
276 linear model with initial species frequencies in 2018, species and their interaction as fixed
277 factors. All models were performed using the packages “lme4” (62) and “car” (63) in R (64).

278

279 **Results**

280 *Are reproductive interactions frequency dependent?*

281 We quantified the number of conspecific and heterospecific claspings from 87 females and
282 mating success of 89 males in our mating trials. We found a significant effect of species
283 frequency on the number of con- and heterospecific claspings of females, but in opposite
284 directions. Females experienced more conspecific claspings when they were common than
285 when they were rare ($\chi^2 = 4.61$, $p = 0.031$; Fig. 2A) but more heterospecific claspings when
286 they were rare than when they were common ($\chi^2 = 11.12$, $p < 0.001$; Fig. 2B). In contrast, we
287 found no effect of species frequency on male mating success. In all the models we found no
288 effect of species nor the interaction between species and frequency (Supplementary Table
289 1B-D).

290

291 *Are reproductive interactions costly and do they result in rare species advantage?*

292 We quantified adult longevity for 128 females (64 of each species) in our mesocosm
293 experiments. We found no main effect of frequency treatment on female longevity nor a
294 significant interaction between species and frequency, suggesting that neither species
295 longevity was affected by changes in the species frequency. However, we found a significant
296 main effect of species on female longevity ($\chi^2 = 24.72$, $p < 0.001$), with shorter longevity (>
297 50%) of *E. cyathigerum* compared to *I. elegans* in these mesocosm cages (Supplementary
298 Table 2B).

299

300 Next, we analysed female productivity (i.e., the number of female offspring in the next
301 generation per adult female in the initial generation) in the mesocosm experiments. Female
302 productivity differed significantly between the two species ($F = 15.63$, $p = 0.028$), with *I.*
303 *elegans* females being on average more productive than *E. cyathigerum*. Importantly, we
304 found a significant and negative effect of species frequency ($F = 53.55$, $p = 0.005$) on female
305 productivity, with lower female productivity in the common compared to the rare frequency
306 treatment (i.e., negative frequency dependence) (Fig. 3). We found no significant interaction
307 between species identity and species frequency (Supplementary Table 2C), suggesting that
308 the strength of negative frequency-dependence was similar in both species. The results were
309 similar when we analysed the total number of emerging individuals in the offspring
310 generation and the number of emerging male offspring (Supporting Table 2D). We found
311 only minor contamination in our control cages, and our results above remain qualitatively
312 similar after correcting for such contamination (Supplementary Analysis 1).

313

314 *Do these species show negative frequency dependence in nature?*

315 Finally, we analysed species density changes across two generations at the 18 natural
316 sympatric sites of *I. elegans* and *E. cyathigerum*. We found a significant effect of initial
317 species frequency (we present results on a logarithmic scale as they show better fit, although
318 untransformed data was also significant) on species density change ($F = 14.95$, $p < 0.001$;
319 Fig. 4). There was no significant effect of species identity nor the interaction between species
320 identity and initial frequency (Supplementary Table 4), suggesting that these two species
321 respond similarly to changes in relative frequencies in nature. These between-generation
322 changes indicate that the higher species frequency was at a site in 2018, the more it declined

323 in abundance the following year. These results suggest negative frequency dependence,
324 consistent with the findings in the mesocosm experiment (Fig. 3).

325

326 **Discussion**

327 Negative frequency dependence is an fundamental requirement for species coexistence, as a
328 species that has a fitness advantage when rare can recover from low abundance and
329 competitive exclusion can be prevented (1, 2). However, our understanding of the underlying
330 mechanisms responsible for negative frequency dependence and stable coexistence is still
331 poor (2, 65). Many previous studies have focused on the ecological niche differences by
332 which negative interspecific interactions can be reduced, although reproductive interactions
333 alone can also cause negative frequency dependence and promote species coexistence (19–
334 22, 32). Our results suggest that intraspecific male mating harassment and the resulting
335 sexual conflict it generates has the potential to reduce female productivity, causing negative
336 frequency dependence and promoting species coexistence. To the best of our knowledge, our
337 study is the first empirical example of how a mechanism not based on ecological niche
338 differences can promote stable species coexistence.

339

340 Sexual conflict can have severe negative effects on female fitness and by extension reduce
341 population growth (23–27, 66–70). Moreover, sexual conflict is expected to increase at
342 higher densities (25, 27, 28, 30), as high densities should increase encounter rates between
343 the sexes and thereby elevate male mating harassment on females (25, 28). If the negative
344 fitness effects of sexual conflict on females are larger when species are common and reduced
345 when rare, sexual conflict could lead to negative frequency-dependence and rescue rare
346 species from competitive exclusion (19, 20). Consistent with these predictions, we found
347 evidence for negative frequency-dependence on female productivity (Fig. 3). Our mating

348 experiments suggest that sexual conflict could be the driving mechanism causing negative
349 frequency dependence. Although other mechanisms (e.g., predation, cannibalism), especially
350 during larvae stage could also influence species relative frequency changes in the wild.
351 Conversely, heterospecific mating attempts are likely to be shorter in duration, given that
352 females reject heterospecific males and given that heterospecific male claspers do typically
353 not match female prothorax structures (71, 72). Therefore, heterospecific mating attempts are
354 likely to be less costly than conspecific male mating attempts.

355

356 We suggest that the loss of female productivity in our mesocosm experiment corresponds to a
357 doubling of male mating harassment, measured as the number of conspecific claspings in
358 both *E. cyathigerum* and *I. elegans* when they are common (Fig. 2A). In our mesocosm
359 experiments, female productivity of *E. cyathigerum* when common was only 17% of the
360 productivity when rare, and in *I. elegans* productivity when common was only 27% of the
361 productivity when rare, demonstrating strong negative frequency dependence of female
362 fitness. Moreover, in damselflies, males remove the sperm from previous copulations, leading
363 to no obvious female benefits from multiple matings (59, 60). Our experimental setup only
364 allowed us to identify if a male attempted to mate a female if he managed to clasp her, but we
365 could not quantify mating attempts that did not result in claspings (i.e., chasing and fighting),
366 nor if there were multiple mating attempts by the same male. Moreover, the effect of male
367 harassment on females might not translate only in loss of fecundity but also reduce larvae
368 survival (73). Therefore, our measure of sexual conflict is likely to be conservative and
369 underestimate the true level of total mating harassment that females are likely to have
370 experienced in our experimental settings and in nature.

371

372 In addition to male mating harassment and sexual conflict in the adult stage, intra- and
373 interspecific competition during the aquatic larval stage could also potentially have decreased
374 the number of adult female emergences. However, a previous study found that under the
375 current environmental conditions, *I. elegans* and *E. cyathigerum* larvae were competitive
376 equivalent (54). Moreover, previous experimental evidence with different *Ischnura* and
377 *Enallagma* species from North America showed no frequency-dependent mortality or growth
378 rate in larvae in the absence of predators (52). In agreement with our mesocosms with no
379 predators (*but see* (50) for an effect of relative frequency in growth rates). However, in
380 natural settings damselfly species coexistence can be achieved by predation during larvae
381 causing negative frequency dependence (9, 52). Moreover, *Ischnura* and *Enallagma* species
382 in North America show striking larval behavioural differences, with *Ischnura* being more
383 active and susceptible to fish or dragonfly predators (53). Given that damselflies in our area
384 occur in a mosaic of lakes with fish or dragonflies as the top predator, as well as in the
385 absence of them, it is likely that different mechanisms could be acting separately or
386 synergistically in the different localities. However, in the absence of top predators, sexual
387 conflict could have a sufficiently large effect to rescue populations from exclusion and
388 promote coexistence.

389

390 We have experimentally investigated how sexual conflict can affect species coexistence and
391 by extension the maintenance of local diversity. Our results point to the importance of
392 mechanisms based on the intrinsic reproductive interactions within species and suggest that
393 sexual conflict can generate negative frequency dependence and promote species coexistence.
394 However, further evaluating the relative importance and interaction of reproductive
395 interactions with other ecological factors, such as the presence and type of predators, would
396 help us better understand the mechanisms promoting species coexistence in nature.

397

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577

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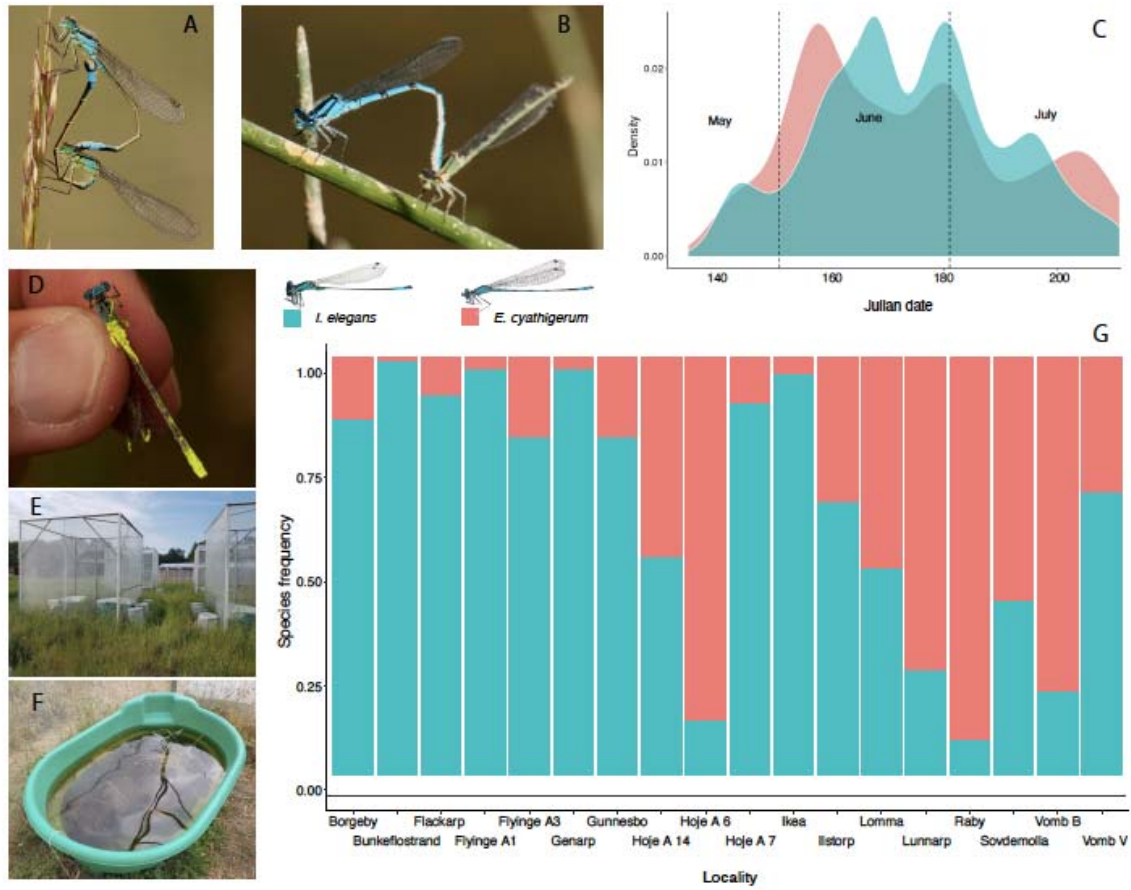
579 **Figure 1.** We used two species of damselflies *I. elegans* (**A**) and *E. cyathigerum* (**B**) to study
580 the role of sexual conflict in species coexistence. These species are ecologically similar, and
581 they overlap during the adult life stage (**C**). We performed mating experiments in small cages
582 in which we marked male claspers and genitalia (**D**) to measure the intensity of different
583 reproductive interactions when species are common and rare. We performed mesocosm
584 experiments in large outdoor cages (**E**) with water containers (**F**) across the full life cycle to
585 measure the costs of reproductive interactions. Finally, we did community surveys in 18 wild
586 populations with different species frequencies (**G**) to estimate how species density changes
587 were affected by initial species frequencies.

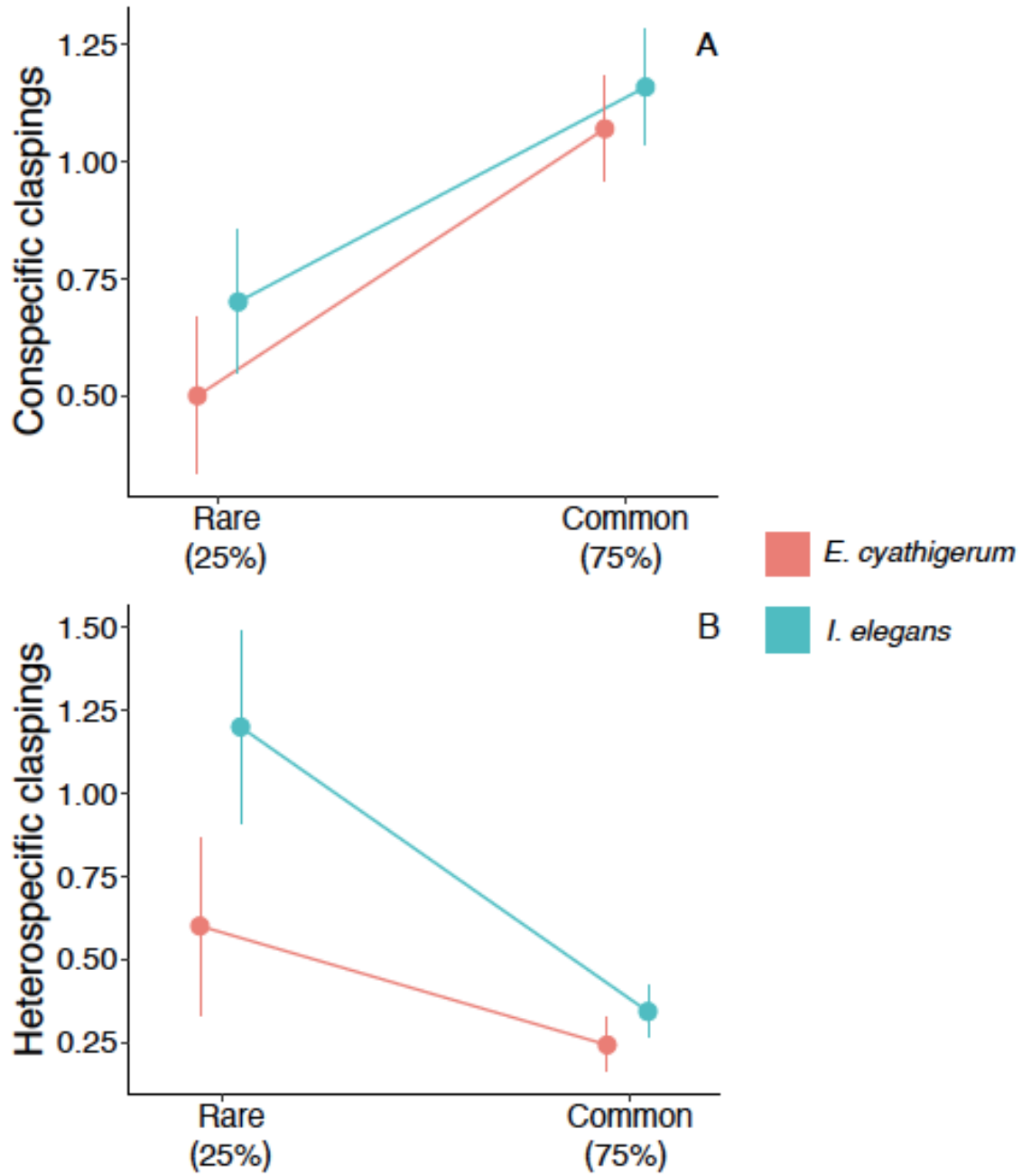
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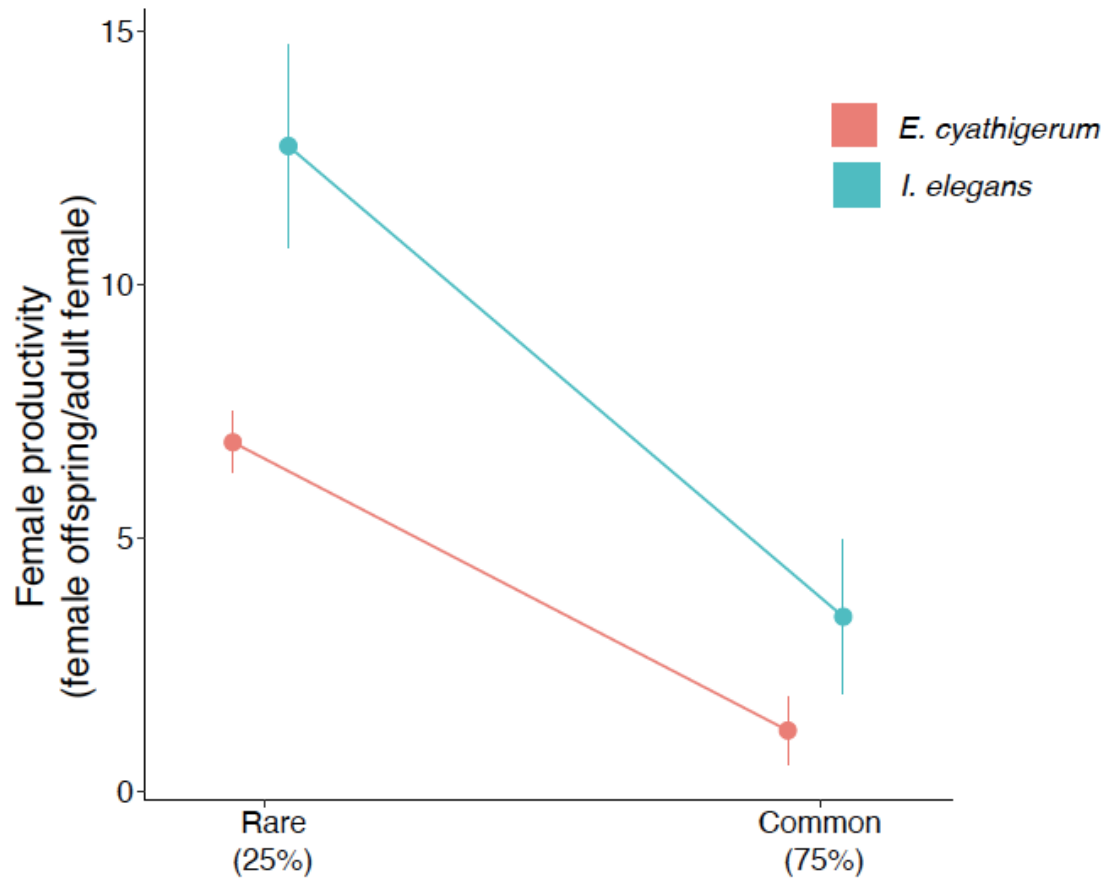
589 **Figure 2.** We perform a series of mating experiments in which we manipulated species
590 frequencies, “rare” (25%) and “common (75%) to test the intensity of intra and interspecific
591 reproductive interactions (Supplementary Table 1). We found that the intensity of sexual
592 conflict, measured as the number of mating attempts (i.e., claspings), was more intense when
593 species were common than when rare (**A**). Heterospecific matings attempts followed the
594 opposite pattern, females experiencing more mating attempts by heterospecifics when rare
595 than when common (**B**). Points show the means and error bars the standard error.
596

597 **Figure 3.** We used mesocosm experiments to quantify the costs of sexual conflict in female
598 fitness. We found strong negative frequency-dependence in female productivity (measured as
599 the number of female offspring that emerge in the following generation per female in the
600 initial generation), having higher fitness when rare over common. Similar results were found
601 when we analyzed total productivity (i.e., number of offspring emerged per adult female in
602 the initial generation; Supplementary Table 2). Points show the means and error bars the
603 standard error.
604

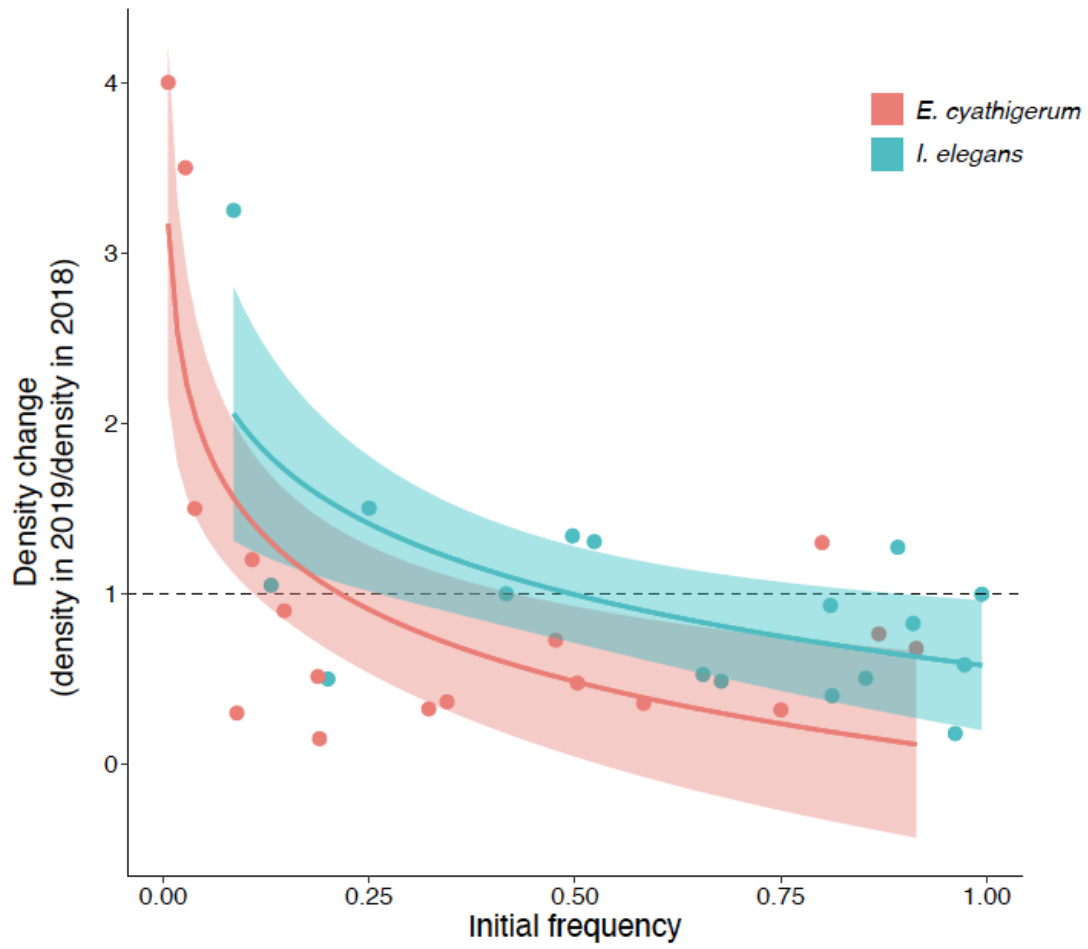
605 **Figure 4.** We monitored damselfly communities in 18 localities during two consecutive years
606 (2018-2019; Supplementary Table 3). We calculated the initial species frequency in 2018 and
607 the species density change (i.e., species density in 2019/ density in 2018). We found evidence
608 for negative frequency dependence and rare species advantage in both *I. elegans* and *E.*
609 *cyathigerum* (Supplementary Table 4). Higher the initial species frequency in 2018 suffer a
610 density decline, lower initial frequencies increase their densities. Points show individuals
611 observations at each locality, line show model predictions (best fit with in a logarithmic
612 regression) and shaded areas confidence interval.
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Supplementary Information

630

Sexual conflict promotes species coexistence through negative frequency dependence

631

Miguel Gómez-Llano, Sofie Nilén, Iain R. Moodie and Erik I. Svensson

632

633

Supplementary Table 1. We performed a series of behavioural mating assays in which we

634

quantified con- and heterospecific mating attempts and male mating success under different

635

frequency treatments. The sample sizes in the different frequency treatments, number of

636

individuals used of each sex and number of replicates are shown in (A). We analysed the

637

effects of species frequency, species and their interaction in conspecific claspings (B),

638

heterospecific claspings (C) and for male mating success (D).

639

A) Species composition and sample sizes in the behavioural mating experiments.

Species frequency	Species	No. males	No. females	Replicates	Tot. males	Tot. females
Common (75%)	<i>I. elegans</i>	3	3	13	39	39
Rare (25%)	<i>E. cyathigerum</i>	1	1		13	13
Common (75%)	<i>E. cyathigerum</i>	3	3	10	30	30
Rare (25%)	<i>I. elegans</i>	1	1		10	10

640

641

B) Number of conspecific male claspings.

	χ^2	df	P
Replicate	0.25	1	0.614
Frequency	4.61	1	0.031
Species	0.44	1	0.504
Frequency : Species	0.08	1	0.772

642

643

C) Number of heterospecific claspings.

	χ^2	df	P
Replicate	0.007	1	0.933
Frequency	11.12	1	<0.001
Species	2.38	1	0.122
Frequency : Species	0.207	1	0.648

644

645 D) Male mating success (mated=1; un-mated=0).

	χ^2	df	P
Replicate	0.34	1	0.559
Frequency	0.271	1	0.602
Species	0.149	1	0.699
Frequency : Species	0.148	1	0.699

646

647 **Supplementary Table 2.** We carried out multi-generational mesocosm experiments in large
 648 outdoor cages with water containers across the entire life-cycle of damselflies (Fig. 1). We
 649 estimated female productivity (number of emerging female offspring in the next generation
 650 per adult female in the initial generation) for the two different species (*I. elegans* and *E.*
 651 *cyathigerum*) under the two different frequency treatments (Rare: 25 % and Common: 75 %).
 652 Sample sizes in the different frequency treatments, number of individuals per replicate and in
 653 total and the number of replicates are shown in (A). We analysed the effect of species
 654 frequency, species and their interaction on female longevity (B), female productivity
 655 (measured as the number of female offspring emerged in the next generation per adult female
 656 in the parental generation in 2018; C), and total female productivity (measured as the total
 657 number of offspring emerged in the next generation per adult female in the parental
 658 generation; D).

659 A) Species composition and sample sizes in the mesocosm experiments.

Species frequency	Species	No. males	No. females	Replicates	Tot. males	Tot. females
Common (75%)	<i>I. elegans</i>	12	6	5	60	30
Rare (25%)	<i>E. cyathigerum</i>	4	2		20	10
Common (75%)	<i>E. cyathigerum</i>	12	6	4	48	24
Rare (25%)	<i>I. elegans</i>	4	2		16	8

660

661 B) Adult female longevity (days).

	χ^2	df	P
Frequency	0.116	1	0.733
Species	24.724	1	< 0.001
Frequency : Species	0.1003	1	0.751

662

663 C) Female per-capita productivity (No. emerging female offspring in 2019 and 2020).

	F	df	P
Frequency	31.68	1	0.004
Species	9.25	1	0.038
Frequency : Species	1.83	1	0.247

664

665 D) Total female per-capita productivity (No. emerging female and male offspring in the
666 next generation per female; data from 2019 and 2020).

	F	df	P
Frequency	18.29	1	0.012
Species	5.11	1	0.086
Frequency : Species	1.2	1	0.333

667

668 **Supplementary Table 3.** We monitored community dynamics at 18 natural sites during the
669 reproductive season of 2018 and 2019, quantifying the relative species frequency and density
670 in both years. Here we provide information about the geographic locations of these different
671 sites.

Locality	Coordinates	Visits in 2018	Visits in 2019
Borgeby	55.738868, 13.047789	3	4
Bunkeflostrand	55.538688, 12.923294	4	5
Flackarp	55.689522, 13.167998	4	5
Flyinge 30 A1	55.745274, 13.359529	5	4
Flyinge 30 A3	55.749590, 13.339951	4	4
Genarp	55.608782, 13.385463	5	5
Gunnesbo	55.734698, 13.153432	4	5
Höje Å 14	55.649639, 13.319925	4	4
Hoje Å 6	55.680208, 13.257543	4	5

Hoje Å 7	55.669535, 13.300354	4	5
IKEA (Malmö)	55.553535, 12.983824	3	4
Ilstorp	55.616181, 13.663855	4	5
Lomma Kyrkdamm	55.684672, 13.085663	4	5
Lunnarp	55.649298, 13.330419	5	5
Råbydammen	55.686356, 13.233741	3	4
Klingavälsån: Sövdemölla	55.601371, 13.657192	4	4
Vombs Bruksgård	55.653859, 13.601517	4	5
Vombs Vattenverk	55.658641, 13.541997	4	5

672

673 **Supplementary Table 4.** Community dynamics in the 18 wild populations was analysed as
 674 the effect of species frequency in 2018 (log-transformed; **A**) and species identity in species
 675 density change (density in 2019/density in 2018) using a general linear model. Non-
 676 transformed species frequency in 2018 show qualitatively similar results (**B**).

677 **A**

	F	df	p
log(Frequency 2018)	14.95	1	< 0.001
Species	0.438	1	0.512
log(Frequency 2018) : Species	0.932	1	0.341

678 Adj. $R^2 = 0.286$

679 **B**

	F	df	p
Frequency 2018	18.39	1	0.041
Species	0.005	1	0.972
Frequency 2018 : Species	5.56	1	0.25

680 Adj. $R^2 = 0.098$

681 **Supplementary Analysis 1.** We found evidence of small contamination of both species in
 682 the control cages with allopatric species frequencies (100 % *I. elegans* or *E. cyathigerum*).
 683 The source of this contamination might have come from larvae or eggs attached to the

684 vegetation used to inoculate the water containers or adult of either species entering the cages
685 by mistake. The mean contamination in the two *E. cyathigerum* cages was 18% (min = 5%,
686 max = 31%), and in the two *I. elegans* cages 2% (min = 0.8%, max = 4%). Assuming similar
687 levels of contamination occurred in the other cages, we performed two corrections to see how
688 this might have confounded our results. First, we removed from the female productivity of
689 each cage the mean percentage of contamination of each species, (-18% and -2% of *E.*
690 *cyathigerum* and *I. elegans*, respectively) (**A**). Second, we performed a more conservative
691 correction, by instead removing the maximum level of contamination found of each species (-
692 31% and -4% of *E. cyathigerum* and *I. elegans*, respectively), (**B**). The two models below (**A**
693 and **B**) were performed using the corrected value of female productivity as response variable,
694 species frequency, species identity and their interaction as fixed factors in a generalized
695 linear model. In both cases the results are qualitatively the same as in the uncorrected
696 emergences and the effects of both “frequency” and “species” remain highly significant (see
697 also Fig. 3).

698

699 A) Corrected analysis of female productivity assuming mean contamination equivalent to
700 the control cages.

	F	df	p
Frequency	28.91	1	0.005
Species	12.88	1	0.022
Frequency : Species	2.97	1	0.159

701

702 B) Corrected analysis of female productivity assuming maximum contamination
703 equivalent to the control cages.

	F	df	p
Frequency	26.72	1	0.006
Species	16.1	1	0.015
Frequency : Species	4.05	1	0.114

704

705

706