1   # Sexual conflict promotes species coexistence through negative

2   # frequency dependence

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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 claspings) 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 claspings per female) on conspecific females in 24 hours. We underscore that this

180   rate of claspings does not take into account mating attempts that did not end up in claspings

181   (i.e., chasing of females) or repeated claspings 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 claspings. 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 m^3) 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 rateunder 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˚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 the18 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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566

574

575   **Authors contributions:** MGL and ES design the study. All authors carried out the

576   experiments. MGL perform the analysis and wrote the text with input from all the authors.

577

578   **Competing**   **interests:**   No   competing   interests

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.

588

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

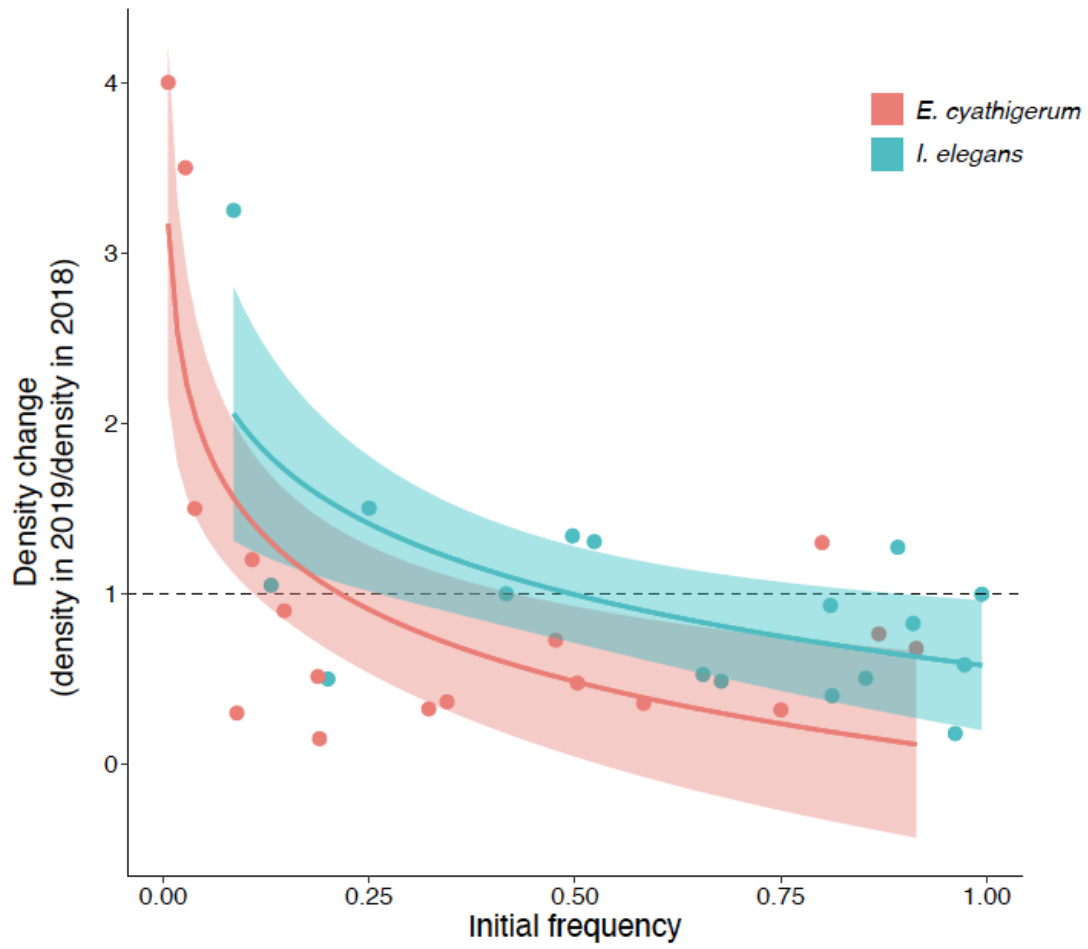612   regression) and shaded areas confidence interval.

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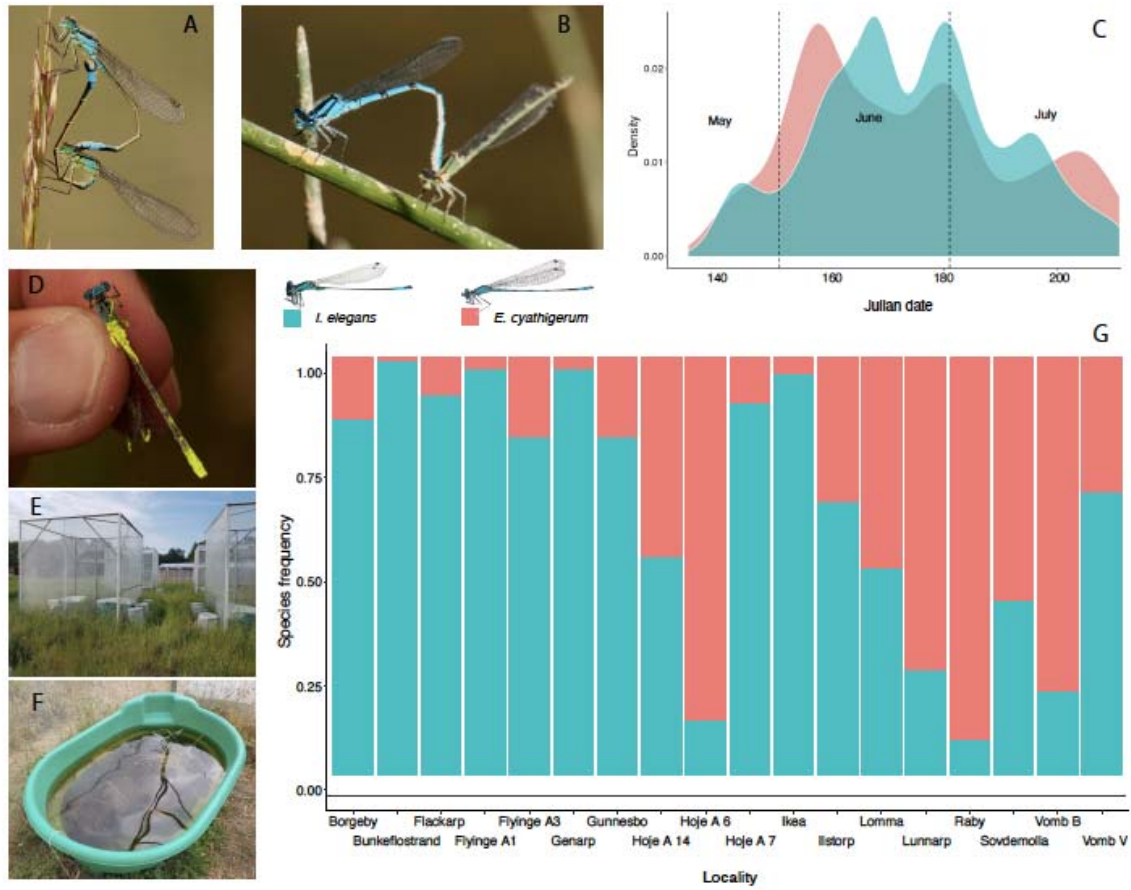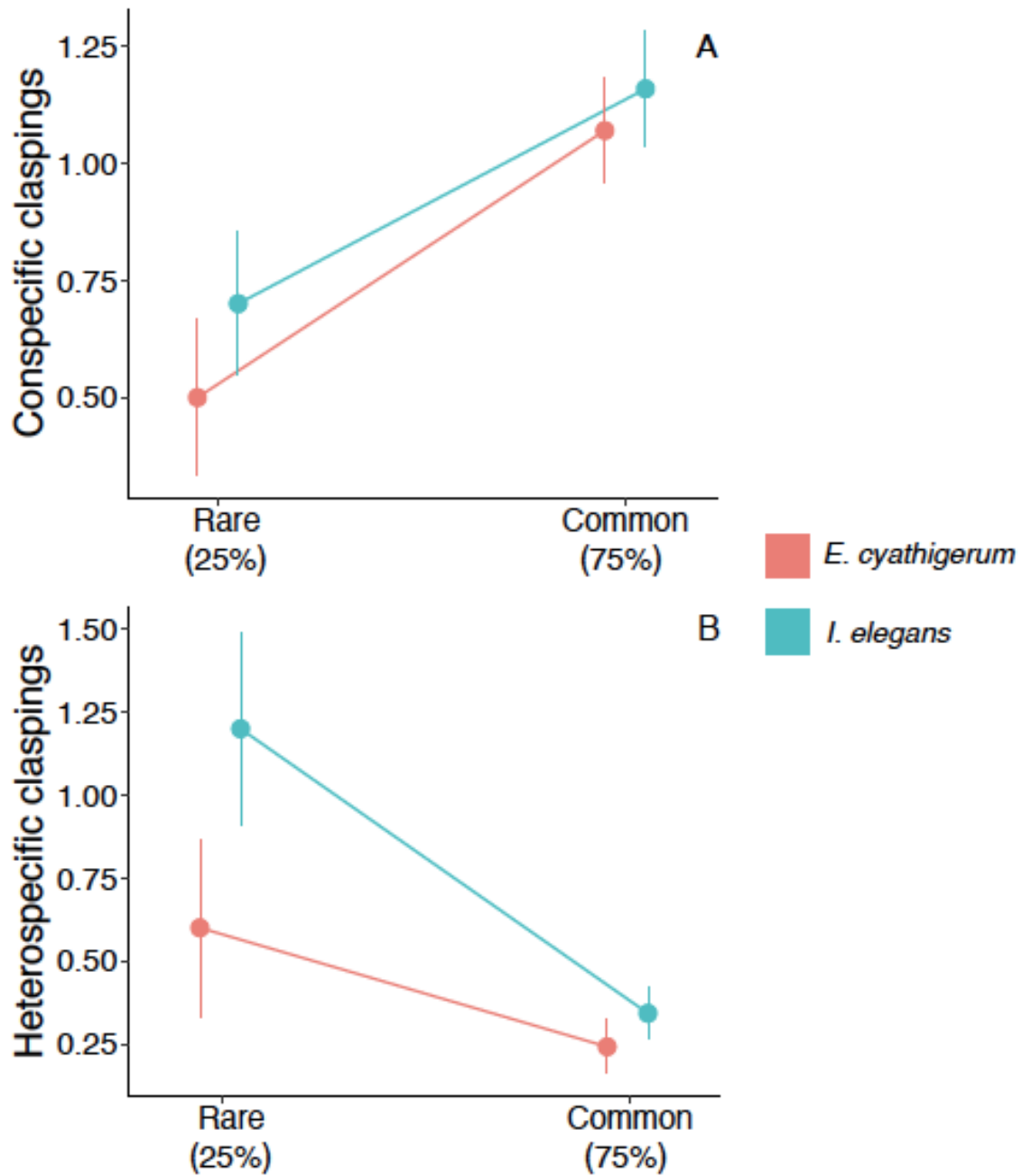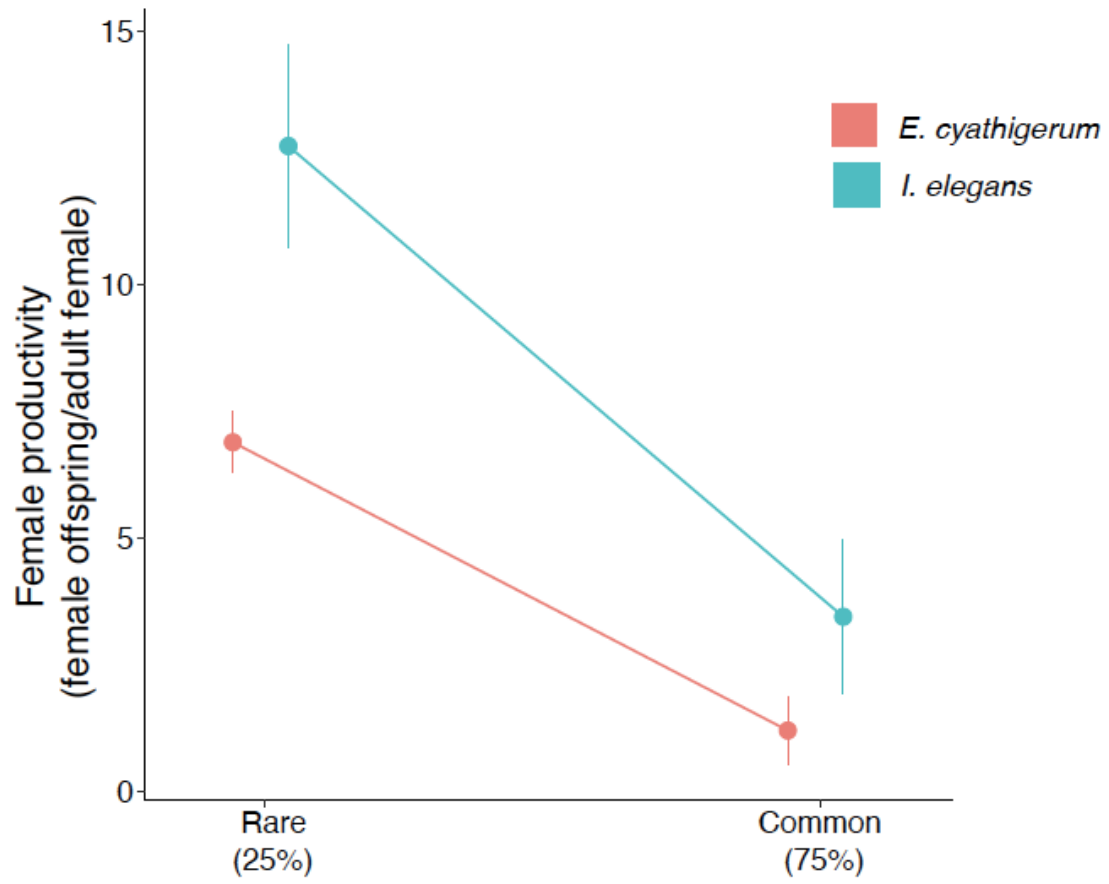
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629   **Supplementary Information**

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. elegans*	3	3	13	39	39
Rare (25%)	*E. cyathigerum*	1	1		13	13
Common (75%)	*E. cyathigerum*	3	3	10	30	30
Rare (25%)	*I. elegans*	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. elegans*	12	6	5	60	30
Rare (25%)	*E. cyathigerum*	4	2		20	10
Common (75%)	*E. cyathigerum*	12	6	4	48	24
Rare (25%)	*I. elegans*	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