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1 **Consequences of resource competition for sex allocation and discriminative behaviors in**
2 **a hyperparasitoid wasp**

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29

30 **Abstract**

31 Population-wide mating patterns can select for equal parental investment in both sexes, but limiting resources,
32 such as mates or developmental substrates, can increase competition leading to biased sex ratios in favor of either
33 sex. Such competition for resources typically occurs in spatially structured populations, where dispersal is limited.
34 Here, we investigate if and how resource competition affects sex allocation, discriminative behaviors and
35 competitive interactions of the wingless hyperparasitoid *Gelis acororum*, which exploits patchily distributed hosts.
36 We show that *G. acororum* sex ratios are male biased and that this is not a consequence of constrained reproduction
37 by virgin females. Our results suggest that this pattern of reproductive investment, which is only rarely observed
38 in parasitoids, is a consequence of resource limitation, in terms of hosts rather than mates. **Further, *G. acororum***
39 **appears not to respond to intrinsic host quality or to prior oviposition in its host.** When competing inter-
40 specifically for host resources, *G. acororum* outcompetes its congener *G. agilis*, but does so mainly when
41 ovipositing on the host first. Overall, our results suggest that host resource limitation could be an important
42 environmental factor shaping sex allocation in *G. acororum*, with competition taking place both intra- and inter-
43 specifically.

44

45 **Keywords**

46 Reproduction; Local mate competition; Local resource competition; Sex ratio; *Gelis acororum*

47 **Introduction**

48 It has been an enduring effort in evolutionary ecology to reveal how organisms' reproductive investment is shaped
49 by their environment. Fisher (1930) was one of the first to explain sex ratio patterns, where equal investment in
50 both sexes is maintained when allocation of resources into either sex conveys similar costs and benefits. Due to
51 frequency dependence, the least common sex is favored by natural selection, ultimately leading to equal sex ratios
52 in subsequent generations. A critical assumption in Fisher's sex ratio theory is population homogeneity, where
53 mate competition is population-wide. In many circumstances, however, populations are structured in terms of
54 mating opportunities. A classic example, local mate competition (LMC), was described by Hamilton (1967) where
55 offspring of only a small number of mothers are confined to the same patch prior to mating and dispersal: male-
56 male competition for mates selects for female biased progeny sex ratios. Alternatively, population heterogeneity
57 can lead to competition for breeding substrates. Clark (1978) was the first to formally describe skewed sex ratios
58 in favor of males in response to severe competition between related females for breeding areas in prosimian
59 primates. Similar findings have been obtained also for other animals, such as marsupials, ungulates, birds and
60 insects (West 2009). Contrary to LMC, it is the female that experiences severe competition for resources; hence
61 local resource competition (LRC) for breeding substrates favors a proportionally higher investment in males over
62 females (Strohm and Linsenmair 1997; Foitzik et al. 2010).

63

64 Patchy environments often increase the probability of encountering kin and interactions between related
65 individuals underlie many theories of sex ratio evolution (West 2009). Restriction to certain patches does not solely
66 involve groups of related individuals and patch exploitation by other, unrelated, individuals is likely quite common
67 when many individuals exploit similar resources (Herre 1985). Indeed, in the context of LRC, Silk (1983)
68 described how male-biased sex ratios were maintained in primates, even when larger group sizes were considered
69 and where individuals faced competition from both related and unrelated conspecifics. Clearly, larger group sizes
70 do not always lead to selection for unbiased sex ratios simply by providing more mating opportunities. On the
71 contrary, competition may become even more intense (Silk 1983). **Males or females may not only have to**
72 **compete with direct relatives, but also with individuals of other species within their community.** Alongside
73 intra-specific competition, inter-specific interactions might further increase competitive pressures for access to
74 similar and limiting resources, potentially distorting sex ratios.

75

76 Competition for resources can shape patterns of reproductive allocation, but it can further affect the extent to which
77 discriminative behaviors are needed to assess the quality or quantity of resources (West et al. 2002). Discriminative
78 behaviors can be highly beneficial. For instance, the negative effects of inbreeding can be avoided when locally
79 mating females can identify kin to avoid sib-mating (Ode et al. 1995; Lihoreau et al. 2007). Furthermore, the ability
80 of a female to estimate the availability or quality of an oviposition substrate is critical for the survival of her
81 offspring and therefore the mother's reproductive success (Godfray 1994). Such benefits are counteracted,
82 however, by substantial energetic costs associated with increased discriminative abilities (DeWitt et al. 1998;
83 Chevin et al. 2010; Auld et al. 2010). In *Drosophila melanogaster*, lines selected for increased recognition of high
84 versus low quality oviposition substrates showed a clear decrease in egg laying behavior compared to controls;
85 hence discrimination ability can negatively affect key fitness-related traits, such as fecundity (Mery and Kawecki
86 2002, 2004). Thus when competition is severe, the costs associated with discriminative abilities might outweigh
87 their benefits, favoring indiscriminate behaviors (West et al. 2002).

88

89 Parasitic wasps are insects whose larvae develop in, or on the bodies of other arthropods (Godfray 1994) and are
90 common model organisms in studying the evolution of sex ratios (Hamilton 1967; Trivers and Willard 1973;
91 Charnov et al. 1981; Werren 1983; Godfray 1994; Somjee et al. 2010; Nelson and Greeff 2011; King and Kuban
92 2012). This is in part because their haplodiploid sex determination offers female parasitoids a choice of investing
93 resources in males (haploid eggs) or females (diploid eggs) (Cook 1993). Discriminative behaviors are also well-
94 documented in hymenopteran parasitoids and nearly all tested species show some ability to discriminate between
95 resources of different qualities, such as mates (Grant et al. 1980; Godfray and Cook 1997; Raychoudhury et al.
96 2010) and hosts (Vinson 1976; Godfray 1994; Vinson et al. 1998; van Baaren et al. 2009). Furthermore, many
97 parasitoid species compete heavily for resources, both within and between species (Price 1972; Harvey et al. 2013).
98 For example, different developmental stages of the host, such as eggs or larvae, often harbor their own parasitoid
99 guilds (Price 1972) and both within and between these guilds competition for host resources can be intense,
100 particularly for solitary parasitoids where only one individual can emerge from a single host (Godfray 1994).

101

102 Here, we study the solitary, wingless hyperparasitoid *Gelis acororum* (Hymenoptera: Ichneumonidae) that
103 commonly attacks patchily distributed gregarious hosts on the ground and in low vegetation. Winglessness reduces
104 the dispersal potential and the patchy distribution of clusters of hosts leads this parasitoid exploit groups of separate
105 hosts together, in a manner termed 'quasi-gregarious' (e.g. Hardy 1992, Godfray 1994). Female-biased sex ratios

106 are prevalent amongst gregarious and quasi-gregarious parasitoids and may result from inbreeding or competition
107 between males for access to mates (LMC), (Godfray 1994, West 2009). To establish whether females avoid
108 inbreeding, we first performed mate choice experiments using related or unrelated males. We then tested for LMC
109 by determining sex allocation patterns, following mating with a related or an unrelated male. In contrast to the
110 female bias often observed due to LMC, sex ratios were male biased. The remainder of our experiments were
111 designed to test candidate explanations for male-biased sex ratios. We first confirmed that similar sex ratio patterns
112 were observed among the progeny of a larger number of females whose mating was not experimentally controlled.
113 We then assessed whether *G. acororum* is able to recognize and discriminate against hosts of different quality and
114 prior parasitism status, as reduced discriminative behaviours are expected under strong competitive pressure.
115 Finally, we determined *G. acororum*'s competitive ability when competing for resources with its congener *Gelis*
116 *agilis*, this allowed us to estimate the competitive pressure posed by a species with which it naturally co-occurs
117 and which utilizes similar host resources.

118

119 **Materials and methods**

120 ***Origin and rearing of insects***

121 The primary endoparasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) and its host *Pieris brassicae*
122 (Lepidoptera: Pieridae) were obtained from existing cultures at the University of Wageningen (WUR, the
123 Netherlands) and were originally collected from agricultural fields near the university. *Cotesia glomerata* cultures
124 were maintained as described in Harvey (2000). *Gelis acororum* and *G. agilis* were collected from *C. glomerata*
125 cocoon clutches placed on a garden plot adjacent to the Netherlands Institute of Ecology in Wageningen, the
126 Netherlands. Cultures were maintained within population cages at a constant temperature of $25 \pm 1^\circ\text{C}$, a 16:8 L:D
127 regime and a relative humidity of $65\% \pm 10$ with access to honey and water on cotton wool. Both species were
128 maintained on 1-2 day-old *C. glomerata* pupae and allowed to host-feed. Host-feeding is essential for egg
129 maturation in *G. agilis* (Harvey 2007; Harvey et al. 2011) and has also been observed in *G. acororum* (Pers. obs.
130 B. Visser, J. Harvey). Similar to *G. agilis*, host-feeding likely enhances the rate of egg maturation in *G. acororum*
131 and could be essential for maintaining egg maturation later in life; hence all *G. acororum* females were allowed
132 to host-feed. For experiments, abiotic conditions were similar to that of the cultures, but all behavioral observations
133 were performed at a temperature of $20 \pm 1^\circ\text{C}$.

134

135 ***Inbreeding avoidance***

136 To obtain individuals of known family status, females of a range of ages were obtained from population cages and
137 placed singly in small Petri dishes ($\emptyset = 6\text{cm}$). Each female was allowed access to a clutch containing approximately
138 25-35 *C. glomerata* cocoons, honey and water on cotton wool for 4 days (approximating the period during which
139 the cocoons are suitable for parasitism). After 1 week of development, unparasitized cocoons from which *C.*
140 *glomerata* had emerged were removed, and cocoons containing *G. acororum* progeny were placed singly in small
141 Petri dishes. Within one week after adult emergence, mate preference for a brother or an unrelated male was tested
142 for 20 host-fed *G. acororum* females. For these mate-choice experiments, a brother and an unrelated male were
143 introduced into a small Petri dish first, followed by introduction of the female. All interactions were videotaped
144 for 24 minutes or until mating was observed, after which mate-choice was determined by tracking individuals.

145

146 ***Sex allocation patterns through local mate or resource competition***

147 To test if females adjusted sex ratios following sib-mating, additional pairs were allowed to mate. A total of 13
148 females that had mated with a brother and 9 females that had mated with an unrelated male were transferred to a
149 large Petri dish ($\emptyset = 12\text{ cm}$) and offered 35 1-2 day old *C. glomerata* cocoons, as well as honey and water on
150 cotton wool for a duration of 4 days. Secondary sex ratios were determined two weeks later, following adult
151 eclosion.

152

153 Sex ratios were further determined for the progeny of 81 host-fed females from population cages, where each
154 female had access to approximately 25-35 *C. glomerata* cocoons, honey and water on cotton wool during 4 days.
155 Cocoon clutches far exceeded the number of cocoons that can maximally be parasitized by *G. acororum* and
156 contained an average of 28 (S.E. ± 0.33) *C. glomerata* cocoons. On average 15 (S.E. ± 0.72) of these cocoons
157 remained unparasitized by *G. acororum*, leading to emergence of the host *C. glomerata*. An average of 4 (S.E.
158 ± 0.30) *G. acororum* offspring developed successfully.

159

160 ***Discriminative abilities***

161 *External host marking and internal host discrimination*

162 In several parasitoid species, females are known to leave a chemical mark on their host after parasitism, lasting up
163 to several hours, and which is typically used to signal the presence of an egg to a conspecific female (Nufio and
164 Papaj 2001). To establish whether *G. acororum* discriminated between cocoons previously parasitized by itself or
165 a conspecific, 22 females obtained from population cages were first placed singly and allowed to parasitize one *C.*

166 *glomerata* cocoon. Following oviposition the cocoon was immediately removed and placed into a small Petri dish
167 together with a cocoon that had been parasitized by an unrelated female. Each female was then introduced into the
168 Petri dish and allowed to choose during 1 hour or until oviposition occurred between a cocoon parasitized by itself
169 or a cocoon parasitized by an unrelated female.

170

171 Unlike species that can detect external marking, some parasitoids can deduce parasitism status of the host only
172 after probing it with the ovipositor, for instance through detecting physical or biochemical changes on the host
173 surface or in the host body itself (King and Rafai 1970). In subsequent experiments, females were offered hosts
174 containing *G. acororum* progeny that had developed until the pre-pupal stage. To obtain cocoons parasitized by
175 *G. acororum*, females from population cages were placed singly in a small Petri dish with access to 1 *C. glomerata*
176 cocoon. Each female was observed for 6 hours or until oviposition occurred. Progeny were then allowed to develop
177 until the pre-pupal stage over the following 7 days. To test whether or not females could discriminate parasitized
178 from unparasitized cocoons, a total of 21 females were allowed to choose between a cocoon containing a *G.*
179 *acororum* pre-pupa and a 1-2 day old unparasitized cocoon containing the host *C. glomerata*.

180

181 *Viability assessment*

182 To determine if females were able to assess viability of the host (i.e. whether it was alive or dead), 1-2 day-old *C.*
183 *glomerata* cocoons were frozen at -18°C for 3 days. Prior to the experiments cocoons were left at room temperature
184 for at least one hour. 19 females were then placed singly in a small Petri dish and offered a choice between a live
185 and dead host for 6 hours or until oviposition occurred.

186

187 *Intra-specific host recognition*

188 To evaluate intra-specific discriminative ability of *G. acororum*, female preference for cocoons containing 7 day-
189 old progeny varying in the level of relatedness was determined using three two-choice combinations: 1) A cocoon
190 containing the female's own progeny vs. a cocoon containing her sister's progeny (n=14); 2) the female's own
191 progeny vs. progeny of an unrelated female (n=18); 3) her sister's progeny vs. progeny of an unrelated female
192 (n=16). For each experiment, a single female was allowed to choose for 6 hours or until oviposition occurred.

193

194 *Competitive abilities*

195 To determine larval survival when *G. acororum* is competing with its sister species *G. agilis*, single females of
196 both species were allowed to oviposit on 1 *C. glomerata* cocoon for 6 hours or until oviposition occurred. Cocoons
197 parasitized by *G. acororum* were then offered to *G. agilis* 1 (n = 46), 3 (n = 31) and 7 days (n = 22) after initial
198 oviposition and observed for 6 hours or until oviposition occurred. **To evaluate competitive ability of *G.***
199 ***acororum* when offspring had a developmental disadvantage (i.e. the competitor is at a more advanced**
200 **developmental stage), reciprocal experiments were also performed, in which *G. agilis* oviposited first,**
201 **followed by *G. acororum* (n = 24, 12 and 18 after 1, 3 and 7 days, respectively).** After two weeks of
202 development, emergence was monitored and species identity recorded.

203

204 *Statistics*

205 Female preferences for an unrelated or related male were determined using a Binomial test. Sex allocation patterns
206 were analyzed using generalized linear models assuming quasi-binomially distributed error variances (logistic
207 analyses for quasi-brood sex ratios) and quasi-Poisson distributed error variances (log-linear analyses for numbers
208 of males per quasi-brood) with significance assessed through model simplification (Crawley 1993; Wilson and
209 Hardy 2002). Meelis tests were used to assess sex ratio variances: the significance of any deviation from
210 binomiality for sex ratio and mortality data was assessed using the test statistic, U . The associated variance ratio,
211 R , was used as a quantification of variance, where overdispersion is indicated by $R > 1$ and underdispersion by R
212 < 1 (Nagelkerke and Sabelis 1991; Krackow et al. 2002). Data on host choice preferences and differential survival
213 in competition experiments were analyzed using Binomial tests. GLM's were performed using the statistical
214 software package GenStat version 15 (VSN International) and binomial tests were performed using R software
215 version 2.12.1 (R Development Core Team 2010).

216

217 **Results**

218 ***Inbreeding avoidance and sex allocation patterns through local mate or resource competition***

219 In mate-choice experiments, females did not show a preference for either a brother or an unrelated male. Out of
220 20 females for which mate-choice was assessed, half chose a brother as a mate, whereas the other half chose an
221 unrelated male (Binomial test: $p=1$). We found no evidence of sex ratio adjustment following sib-mating ($F_{1,20} =$
222 $1.69, p = 0.210$), no relationship between sex ratio and quasi-brood size ($F_{1,19} = 0.15, p = 0.703$, Fig. 1a) nor an
223 interaction between these main effects ($F_{1,18} = 0.16, p = 0.697$). The number of males per quasi-brood increased
224 significantly with increasing quasi-brood size ($F_{1,20} = 33.74, p < 0.001$, Fig. 1c). Quasi-brood sex ratios were

225 overdispersed (quasi-broods >1 , $n=19$, $U = 4.43$, $p < 0.001$, $R = 3.924$) and strongly male-biased (mean proportion
226 of males: 0.810 , $+SE=0.053$, $-SE=0.068$). Our results further showed that 55% of all females produced single-sex
227 male quasi-broods, 9% produced female-only quasi-broods and 36% produced mixed-sex quasi-broods.

228

229 A total of 331 individuals emerged out of 81 quasi-broods of females from population cages: 221 males and 110
230 females. Sex ratios were male-biased (mean proportion of males: 0.668 , $+SE=0.044$, $-SE=0.047$). Sex ratio
231 variance was overdispersed when all quasi-broods >1 were considered ($n = 69$, $U = 8.61$, $p < 0.001$, $R = 2.775$)
232 but did not differ significantly from binomiality when data from only mixed sex quasi-broods were considered (n
233 $= 25$, $U = -0.89$, $p > 0.05$, $R = 0.865$). For all 81 quasi-broods, there was no relationship between sex ratio and
234 quasi-brood size ($F_{1,79} = 0.01$, $p = 0.926$, Fig. 1b) and a similar result was found for mixed sex quasi-broods only
235 ($F_{1,24} = 0.19$, $p = 0.668$). For both sets of data, the number of males increased significantly with increasing quasi-
236 brood size (all quasi-broods: $F_{1,79} = 68.46$, $p < 0.001$, Fig. 1d; mixed sex quasi-broods: $F_{1,24} = 18.71$, $p < 0.001$).
237 Among all quasi-broods, 51% of females produced single-sex male quasi-broods only, 17% produced female-only
238 quasi-broods and 32% produced mixed-sex quasi-broods.

239

240 ***Discriminative abilities***

241 *External host marking and internal host discrimination*

242 When females were offered a choice between cocoons recently parasitized by themselves or an unrelated female,
243 13 out of 22 females chose their own cocoon over an unrelated cocoon (Binomial test: $p=0.524$), suggesting that
244 *G. acororum* does not mark its host to avoid self-parasitism. Females also did not prefer unparasitized over
245 parasitized cocoons: 10 females oviposited in unparasitized cocoons, while 11 females oviposited in parasitized
246 cocoons (Binomial test: $p = 1$; Fig. 1a).

247

248 *Viability assessment*

249 Choice tests evaluating the ability of *G. acororum* to assess host viability showed that 10 out of 19 females
250 oviposited on dead *C. glomerata* cocoons (Binomial test: $p = 1$; Fig. 1a); hence *G. acororum* females do not
251 discriminate against, or avoid ovipositing on, dead hosts.

252

253 *Intra-specific host recognition*

254 Females did not discriminate between cocoons containing progeny that varied in the degree of relatedness. Eight
255 out of 14 females chose their own cocoon over that of their sister's (Binomial test: $p = 0.791$), 7 out of 18 preferred
256 their own cocoon over that of an unrelated female (Binomial test: $p = 0.481$) and 8 out of 16 females chose their
257 sister's cocoon when offered a choice between their sister's cocoon and that of an unrelated female (Binomial test:
258 $p = 1$; Fig. 1b).

259

260 ***Competitive abilities***

261 Survival of *G. acororum* was higher than that of *G. agilis* when *G. acororum* was the first to parasitize a common
262 host cocoon: The percentage of *G. acororum* individuals surviving to adulthood increased from 74 to 77 and 91%
263 as time elapsed between oviposition events increased (1, 3 and 7 days, respectively: Binomial tests: 1 day $p <$
264 0.01 , $df = 46$; 3 days $p < 0.01$, $df = 31$; 7 days $p < 0.001$, $df = 22$; Fig. 2a). In contrast, when *G. agilis* oviposited
265 first, we found either no difference in survival (after 1 day: $p = 0.839$, $df = 24$), a competitive advantage for *G.*
266 *agilis* (after 3 days: $p = 0.039$, $df = 12$) or higher survival into adulthood for *G. acororum* (after 7 days: $p < 0.001$,
267 $df = 18$; Fig. 2b).

268

269 **Discussion**

270 Mate-choice experiments did not reveal a preference of females in terms of mating with their brother or an
271 unrelated male. Avoidance of sib-mating can reduce the negative effects associated with inbreeding, such as
272 increased haploid male unviability (Heimpel and de Boer 2008); hence inbreeding avoidance could be beneficial.
273 We did, however, not find evidence that *G. acororum* females avoid inbreeding through mate-choice. Females
274 further did not adjust sex ratios in favor of female offspring once they had mated with a brother. Sex ratio
275 adjustment in favor of females could be expected if males that disperse post-mating are faced with intense
276 competition over access to females (Hamilton 1967; Macke et al. 2011). We thus found no evidence for LMC in
277 *G. acororum* and the absence of inbreeding avoidance and LMC suggests that genetic effects of inbreeding might
278 be minimal in this species or that *G. acororum* outbreeds sufficiently to avoid the negative consequences of
279 inbreeding.

280

281 *G. acororum* sex ratios are biased in favor of males; yet most theoretical models on optimal sex allocation patterns,
282 particularly in parasitoids, predict female-biased sex ratios (Hamilton 1967; Charnov et al. 1981; Werren 1983;
283 West 2009). Several conditions may, however, promote a higher investment in males, such as host limitation

284 through dense rearing conditions (Heimpel and Lundgren 2000; Ode and Heinz 2002) and a high number of
285 foundresses (Hamilton 1967; Werren 1983; Debout et al. 2002). It seems unlikely that these factors contributed to
286 the male-biased sex ratios we observed, first because *G. acororum* had only been reared in the laboratory for a
287 limited number of generations and population cages were provided with a surplus of hosts. Second, females were
288 allowed to oviposit on a host cocoon singly during experiments; hence foundress number was fixed at one.
289 Furthermore, under high host or parasitoid density, even if males can become more numerous within the population
290 (i.e. sex ratios increase), the overall sex ratio typically remains female-biased, in contrast to our observations on
291 *G. acororum* where the majority of individuals are males.

292

293 Haplodiploidy in parasitoids allows females to manipulate investment in each sex through the decision of whether
294 or not to fertilize an egg; hence females can adjust the sex ratio of their offspring at the time of oviposition. An
295 important factor affecting sex ratio decisions in parasitoids is host size or quality; typically, mothers preferentially
296 invest in female offspring when hosts are larger (Charnov et al. 1981; Tanaka et al. 1992; Ueno 1997; Ode and
297 Heinz 2002). Male-biased sex ratios are, however, maintained when *G. acororum* oviposits on a larger host species,
298 *Cotesia rubecula*, even though *G. acororum* preferred this host over *C. glomerata* (Pers. Obs. B. Visser, J. Harvey).
299 Alternatively, males can become more abundant through differential mortality of the sexes, even when females
300 invest equally in both sexes during oviposition (Hardy 1992; King 1993; Hardy et al. 1998). Mechanisms that
301 promote sex-specific mortality include inter-sexual conflict (Kapranas et al., 2011) and the potential for increased
302 exposure of detrimental mutations in haploids (Nagelkerke and Hardy 1994). In sexually size dimorphic
303 parasitoids, females can experience higher mortality in smaller or lower quality hosts, leading to a higher number
304 of males (Charnov et al. 1981; Godfray 1994). Size does, however, not differ between the sexes in *G. acororum*
305 (Pers. obs. B. Visser, J. Harvey) making it unlikely that survival of the sexes is differentially affected by host size
306 in this species.

307

308 Male-biased sex ratios in *G. acororum* suggest that LRC might play an important role in this species, where
309 females compete heavily for access to host resources. Over 50% of all quasi-broods produced by *G. acororum* are
310 single-sex and male-only. Single-sex male and female quasi-broods have also been observed in several parasitoid
311 species within the genus *Achrysocharoides* (West et al. 1999). For example, *A. zwoelferi* produces single-sex
312 clutches only (West et al. 2001). The oviposition strategy of this wasp is exceptional, however, because brood
313 sizes are small and male-only broods have an invariable clutch size of 1. Single-sex nests were also observed in

314 the sphecid *Trypoxylon malaisei*, although these constituted only 5% of nests observed (Oku and Nishida 2001).
315 The most straightforward explanation for the occurrence of male-only broods in *G. acororum* would be that
316 females remained unmated (Hardy et al. 1998). However, mating was observed during experiments testing for sex
317 ratio adjustment through LMC; hence lack of mating does not explain the common production of male-only *G.*
318 *acororum* broods. We have yet to identify which conditions affect the decision-making process of females in this
319 species and what the adaptive significance is of producing male-only broods.

320

321 Skewed sex ratios in favor of males can also result when mothers are constrained in sperm usage, for instance
322 when the efficiency of sperm transfer or storage is reduced. In such scenarios, mated females may not be able to
323 fertilize sufficient eggs, leading to a predominance of males. In some parasitoids a single mating suffices to assure
324 continued production of females, despite a reduction in sperm numbers stored over time (Bressac and Chevrier
325 1998), whilst others require multiple matings to avoid sperm depletion (Ode *et al.*, 1997). Our comparison of sex
326 ratios between recently emerged females and population-cage females of a range of ages showed similar sex ratio
327 patterns (Fig. 1), suggesting that sex ratios and the number of male-only quasi-broods produced is are not
328 dependent on female age. Whilst population-cage females are capable of re-mating, female *G. acororum* with
329 limited mating opportunities early during adult life have been observed to produce daughters at more advanced
330 ages (Pers. Obs. B. Visser, J. Harvey), suggesting that sperm depletion plays little or no role in the sexual
331 composition of quasi-broods.

332

333 Individuals can avoid LRC, for instance through dispersal (Silk and Brown 2008; Guillon and Bottein 2011). A
334 clear example can be found in the parasitoid *Mellitobia australica* (Innocent et al. 2010), a species that has two
335 distinct female morphs: one that is better equipped for dispersal (longer wings), and the other better suited for
336 remaining in the natal patch (shorter wings). Indeed, when intra-specific competition for resources increases, *M.*
337 *australica* females invest more heavily in long-winged dispersing morphs. However, when individuals compete
338 with relatives, conspecifics (Clark 1978; Silk 1983; Silk 1984; Silk and Brown 2008) as well as other species,
339 dispersal is unlikely to relieve competitive pressures when community composition remains relatively stable.
340 Winglessness in *G. acororum* reduces its dispersal potential in the field, and unlike males that compete solely with
341 conspecifics for access to mates, females are faced with competition for resources with other parasitoid species
342 that share the same hosts (Harvey et al. 2011; 2013). Within stable dense communities, competition can thus be

343 hard to avoid, and multi-species interactions can further contribute to the distorted sex ratios observed in *G.*
344 *acororum*.

345

346 Females did not show an egg-laying preference for parasitized or unparasitized hosts, hosts that were alive or dead,
347 or cocoons containing progeny varying in the level of relatedness. We thus found no evidence of host and intra-
348 specific discrimination, or an ability to assess host viability. In hymenopteran parasitoids, a complete absence of
349 discriminative behaviors has only rarely been described and such findings have often been questioned or refuted
350 (Lenteren et al. 1978; van Alphen and Visser 1990). Absence of discriminative behaviors in *G. acororum* might
351 suggest that certain host types are rarely encountered in nature, and that selection regimes have not been rigid
352 enough to select for the avoidance of unsuitable hosts. Sait et al. (1996) found that the solitary endoparasitoid,
353 *Venturia canescens*, was unable to discriminate between healthy host caterpillars (*Plodia interpunctella*) and
354 caterpillars heavily infested with a granulosis virus, and readily oviposited into the latter. However, parasitoids
355 were unable to survive to eclosion in hosts exhibiting latent viral infection. *Gelis acororum* does discriminate when
356 offered a choice between a *C. glomerata* cocoon and a cocoon of the larger host *C. rubecula*, where the latter is
357 favored for oviposition (Harvey et al. in prep). *Gelis acororum* thus seems capable of determining either host
358 species identity or marked size differences, but ceases to actively discriminate against different host states when
359 only *C. glomerata* cocoons are available.

360

361 *Gelis acororum* outcompeted its congener *G. agilis* when allowed to oviposit first. When *G. agilis* oviposited first,
362 survival of both species was similar after 1 day, in favor of *G. agilis* after 3 days but reversed in favor of *G.*
363 *acororum* after 7 days. Both species are wingless and share similar resources. *G. acororum* has a high abundance
364 in the field (Harvey et al., in prep) and this is reflected by the success with which it outcompetes its main competitor
365 *G. agilis*. While this study is the first to investigate competitive interactions between sibling species in the genus
366 *Gelis*, a previous study on *G. agilis* showed that it outcompetes the more specialized solitary pupal hyperparasitoid,
367 *Lysibia nana* (Hymenoptera: Ichneumonidae). After more than 72 hours after initial oviposition, *G. agilis* always
368 dominates, leading to a trophic switch for this species in which it can successfully exploit another hyperparasitoid
369 as a host later in the season (Harvey et al. 2011). The timing of oviposition is critical for survival and competitive
370 success in *G. acororum* and in the field temporal variability in host availability and number of competitors are
371 expected to pose major competitive challenges for both species of *Gelis*.

372

373 The male-biased sex ratios observed in *G. acororum* are very unusual among parasitoids, and a combination of
374 low discriminative behaviors and high competitive pressure indicate the possibility of extensive resource
375 competition between females in the field. Such interactions likely occur between conspecifics, but within dense
376 communities where many co-occurring species exploit similar resources, females may also face adverse conditions
377 that are imposed by females of other species. These considerations are lacking in current theories concerned with
378 sex ratio evolution, yet community composition, of both parasitoids and of hosts (Kraft & van Nouhuys 2013)
379 might substantially affect resource availability and inter-specific competition, consequently affecting key traits,
380 such as reproductive success mediated by sex allocation decisions.

381

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385

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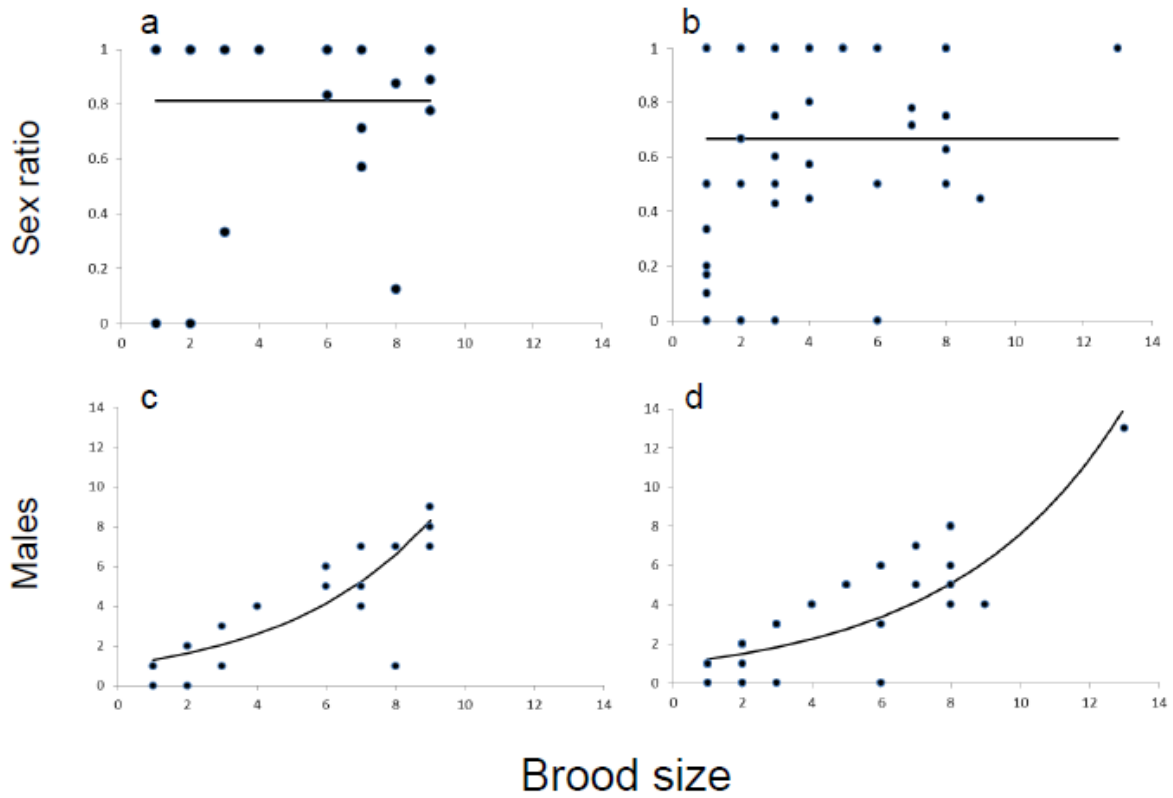
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- 499
- 500

501 **Figures**

502



503

504 **Fig. 1:** Sexual composition of *Gelis acrorum* quasi-broods. Sex ratios are shown from the mate choice experiment

505 quasi-broods (a) and from quasi-broods from population cages (b). The relationship between the number of males

506 and quasi-brood size is shown for the mate choice experiment (c) and for all population cage quasi-broods (d). On

507 all panels regression lines represent the minimal adequate statistical model (logistic analyses, panels a and b; log-

508 linear analyses, panels c and d).

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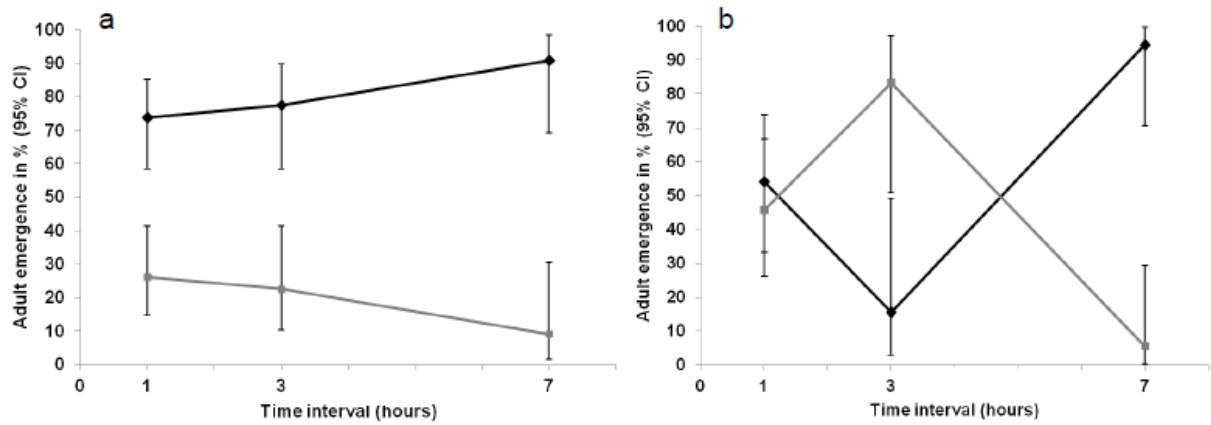
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517 **Fig. 2:** Outcome of intrinsic competition (in percentage of adults that emerged including Wilson's 95% Confidence
518 Interval with continuity correction) between *G. acororum* (black dots) and *G. agilis* (gray dots) 1, 3 and 7 days
519 after initial oviposition with either *G. acororum* parasitizing first (a) or *G. agilis* parasitizing first (b).

520

521