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Keep calm, we know each other: kin recognition affects aggressiveness and conflict resolution in a solitary parasitoid

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1 **Keep calm, we know each other: kin recognition affects**
2 **aggressiveness and conflict resolution in a solitary**
3 **parasitoid**

4

5 **Abstract** - Intraspecific competition for indivisible resources can trigger the expression of
6 agonistic behaviour in individuals of many animal species. Aggressiveness and conflict
7 resolution may be influenced by the value individuals place in the resource (subjective resource
8 value) but also by genetic relatedness between competitors. The ability to differentiate
9 genetically related from unrelated individuals (i.e., kin recognition) can play a key role in the
10 dynamics of agonistic interactions between individuals. In this context, the theory of kin
11 selection predicts that competitors should display fewer aggressive behaviours towards closely
12 related individuals. Recognition of kin conspecifics can be driven by the perception of (1)
13 genetically-linked phenotypic cues (phenotypic matching) and/or (2) environmental cues
14 (familiarity). In the hymenopteran solitary parasitoid *Eupelmus vuilleti*, individuals develop on
15 larvae and pupae of their host, *Callosobruchus maculatus*, which infest cowpea seeds (*Vigna*
16 *unguiculata*). *Eupelmus vuilleti* females can fight for the host on which they lay their eggs.
17 Here, we investigated the effect of genetic relatedness (genotype) and familiarity (the seed
18 containing the host on which they develop as juvenile) on aggressiveness and contests outcome
19 over hosts in *E. vuilleti* females. We first demonstrated that the probability of a conflict
20 escalating was affected by the interaction between genetic relatedness and familiarity among
21 females. We then found that familiarity alone affected the likelihood of contest resolution. The
22 occurrence of escalated conflicts was reduced between related and familiar females, and
23 contests were more likely to be clearly resolved when occurring between familiar competitors.

24 Our results highlighted a parasitoid wasp's abilities to identify and discriminate kin, showing
25 for the first time that two kin recognition components can interact in mediating competition
26 avoidance for resource access in a solitary insect species.

27 **Key words:** *Eupelmus vuilleti*, familiarity, genetic relatedness, phenotype matching, subjective
28 resource value.

29

30 INTRODUCTION

31 Intraspecific competition for an indivisible resource can lead to direct interactions
32 between individuals (i.e., conflicts, Hardy & Briffa, 2013). To prevent other individuals from
33 accessing the resource, animals often exhibit agonistic behaviour during contests (Hack et al.,
34 1997; Riechert, 1998). Winning a conflict can improve access to sexual partners (West-
35 Eberhard, 1979), food resources (Vogel, 2005), territories (Festa-Bianchet et al., 1990) or
36 dominance status (Chase, 1974), but being aggressive can potentially be costly in terms of
37 energy expenditure (Maynard Smith, 1974), physical injuries (Palombit, 1993; Innocent et al.,
38 2011), or increased predation risk (Jakobsson et al., 1995). Animals should therefore evaluate
39 the costs and benefits of exhibiting agonistic behaviours and adjust their behaviour accordingly
40 (Maynard Smith & Price, 1973). In the case of dyadic interactions, game theory predicts that
41 contest outcome should be influenced by the asymmetries that exist between contestants in
42 terms of their intrinsic ability to obtain and hold access to a resource ('Resource Holding
43 Potential', RHP; Maynard Smith, 1974; Parker, 1974), and the value they place on the resource
44 ('Subjective Resource Value', SRV; Maynard Smith & Parker, 1976; Hammerstein, 1981;
45 Enquist & Leimar, 1987). Individuals with higher RHP (e.g., bigger contestants) typically win
46 the resource (Maynard Smith, 1982; Elias et al., 2008; Arnott & Elwood, 2009), and individuals
47 with higher SRV (e.g., with greater egg load or lower food intake) are expected to be more

48 motivated to fight and have a higher probability of winning contests (Stokkebo & Hardy, 2000;
49 Dissanayake et al., 2009). Moreover, these factors have been shown to influence
50 aggressiveness, the most aggressive contestants being generally more likely to win conflicts
51 (Goubault et al., 2007; Mohamad et al., 2010; Mathiron et al., 2018).

52 Aggressiveness during conflict can also vary with the degree of genetic relatedness
53 between competitors (West et al., 2002). Kin selection theory postulates that a trait that does
54 not provide a direct fitness benefit (or to some extent, a negative effect) to the individual who
55 expresses it, can be selected for if it improves the fitness of the individuals carrying the same
56 genes as the protagonist (i.e., indirect fitness; Hamilton, 1964). In the context of conflicts, it
57 would be advantageous for an individual to be less aggressive towards genetically related
58 conspecifics (Hamilton & May, 1977; Griffin & West, 2002; Lizé et al., 2012) because this
59 would maximize their inclusive fitness (West et al., 2001). However, avoiding conflicts
60 between relatives implies kin recognition, i.e. the identification (neurophysiological process),
61 and discrimination (the expression of different behaviours) of genetically related individuals.
62 Two types of recognition exist: (i) recognition based on the perception of genetically encoded
63 phenotypic indices (Gadagkar, 1985) and (ii) familiarity, which refers to the recognition of
64 individuals regardless of their genetic relatedness (Lizé et al., 2012). For instance, two
65 individuals can be considered familiar if they meet often, or if they come from the same
66 environment. The ability to recognize and discriminate genetically related individuals has been
67 observed in various animal taxa, such as nematodes (Kapranas et al., 2016), anuran amphibians
68 (Blaustein & Waldman, 1992) and mammals (Heth et al., 1998). In social insects, for example,
69 kin recognition and avoidance of competition between relatives have been demonstrated in
70 many species, like in sweat bees *Lasioglossum zephyrum*, honey bees *Apis mellifera* (West-
71 Eberhard, 1989), and in the socially polymorphic ant, *Pheidole pallidula* (Fournier et al., 2016).
72 In non-social insects, on the other hand, this phenomenon seems rarer (for reviews, see

73 Fellowes, 1998 and Griffin & West, 2002) or even absent, like in the parasitoid wasps *Aphidius*
74 *matricariae* (Bourdais & Hance, 2009) and *Cotesia glomerata* (Ruf et al., 2010). Theoretical
75 models also predict that kin discrimination may not evolve in populations with a low dispersion
76 of individuals due to the high risk of competition for resource between relatives (West et al.,
77 2002).

78 In hymenopteran parasitoids (i.e. organisms that feed and develop at the expense of a
79 host, resulting in its death), females may compete to feed and lay their eggs on (i.e.
80 ectoparasitoid) or in (i.e. endoparasitoid) a host. Because offspring survival directly depends on
81 the mother's ability to acquire and defend the host (Petersen & Hardy, 1996), agonistic
82 interactions may occur between females that may, in some cases, be fatal, as described in some
83 *Melittobia* species (Godfray, 1994; Hartley & Matthews, 2003; Innocent et al., 2011). In this
84 context, four main factors acting on RHP and/or SRV appear to influence conflict resolution:
85 (1) relative size and age of the opponents, (2) order of arrival on the resource (resident or
86 intruder), (3) egg load, and (4) previous experience. Bigger and older females usually win
87 contests (Petersen & Hardy, 1996; Humphries et al., 2006; Tsai et al., 2014), as do residents
88 (Maynard Smith & Parker, 1976; Enquist & Leimar, 1987), even if they are smaller than
89 intruders (Bentley et al., 2009). In addition, individuals with a greater egg load are generally
90 advantaged during combat; these females value the resource more because a greater egg load
91 would allow them to oviposit on the host sooner (Stokkebo & Hardy, 2000; Mohamad et al.,
92 2010; but see Goubault et al., 2007). Finally, females that have experienced low quality hosts
93 or low host densities are usually more aggressive and win more often in the presence of high
94 quality hosts (Mohamad et al., 2010; Mathiron et al., 2018). Although the ability to treat kin
95 differentially may influence the dynamics of contests between parasitoids competing for hosts,
96 this has been rarely tested empirically. To our knowledge, only two studies measured the effect
97 of kin recognition on competition avoidance and aggression in hymenopteran parasitoids: (1)

98 in the polyembryonic wasp (i.e. clonal multiplication of eggs within the host) *Copidosoma*
99 *floridanum* (Giron et al., 2004) and (2) in the gregarious (i.e. several larvae develop on the same
100 host) parasitoid *Goniozus legneri* (Lizé et al., 2012). In the latter study, Lizé et al. (2012)
101 showed that adult females recognized their relatives through phenotypic cues and familiarity
102 (development on the same host), adjusting their aggressiveness accordingly. Familiarity would
103 thus act as a proxy for kinship. However, no study has measured the effect of genetic relatedness
104 on aggression and conflict resolution in a solitary parasitoid species.

105 Here, we explored the effect of kin recognition on aggressiveness and resolution of
106 conflict in females of the solitary ectoparasitoid wasp, *Eupelmus vuilleti* (Hymenoptera:
107 Eupelmidae). Females parasitize and feed upon larvae and pupae of the cowpea seed beetle
108 *Callosobruchus maculatus* (Coleoptera: Bruchidae), which infest seeds of *Vigna unguiculata*
109 (Fabaceae). *Eupelmus vuilleti* is a solitary parasitoid, which implies that only one individual
110 develops per host (supernumerary larvae are eliminated by larval competition; Fisher, 1961).
111 When several females are simultaneously present on a patch, they tend to protect the host by
112 directing agonistic behaviours towards conspecific competitors (Mohamad et al., 2010;
113 Mathiron et al., 2018). In sub-Saharan Africa, farmers traditionally stored cowpea seeds in
114 granaries, sometimes resulting in populations of both hosts and parasitoids reaching high
115 densities. Since seeds often contain several hosts, several individuals of *E. vuilleti* can emerge
116 from the same seed, leading females to experience intense intraspecific competition with both
117 kin and non-kin conspecifics.

118 We therefore tested whether wasps are able to adjust their aggressiveness during
119 contests for host access depending on their level of genetic relatedness with their competitor.
120 Because reducing the costs associated with conflicts between kin may be beneficial in terms of
121 inclusive fitness, we expected a decrease in aggressiveness and conflict escalation between
122 related females. Moreover, if kin recognition is effective in *E. vuilleti* females, this recognition

123 might be based on phenotypic matching, familiarity, or both. Females may use cues coming
124 from genetically encoded phenotypic traits and/or from a common environment. For example,
125 the seed containing hosts on which wasps developed may itself act as a way to identify kin.
126 Using a full factorial design, we made genetically related or unrelated juveniles develop on
127 hosts within the same seed (familiar) or different seeds (unfamiliar). We then investigated the
128 effects of (1) genetic relatedness among adult females (genetically related vs. unrelated), (2)
129 the seed containing the hosts on which females developed (same vs. different seeds, i.e. familiar
130 vs. unfamiliar), (3) the absolute difference in SRV (number of ready-to-lay eggs) of individuals,
131 and (4) the interaction between these variables on aggressiveness, contest escalation and
132 resolution. As contests are predicted to be clearly settled and less aggressive when contestants
133 show a large difference in SRV (Maynard Smith & Parker, 1976), we expected contests in
134 which the absolute difference in egg load between contestants was large to be less aggressive
135 and often won by the most gravid female. If females recognize kin and avoid fighting against
136 them, this effect of SRV asymmetry should be stronger between unrelated/unfamiliar females.
137

138 **MATERIAL AND METHODS**

139 *Laboratory breeding*

140 We tested individuals of *E. villetti* that were collected on cowpea seeds from crops
141 cultivated in Togo in 2007. Wasps were brought to the laboratory (Institut de Recherche sur la
142 Biologie de l'Insecte, Université de Tours, France) and reared on juvenile stages of *C.*
143 *maculatus*, as described by Jaloux et al. (2004). We performed all experimental procedures in
144 a climate room (temperature = 30 °C and photoperiod = 12:12 h light: dark).

145 *Genotypes*

146 To study the influence of genetic relatedness between females on competition for host
147 access, we bred four genetically inbred strains. Working on individuals coming from more than
148 two genotypes minimized the risk of bias due to contest behaviours specific to those genotypes.
149 To do so, we separately introduced four mating pairs from the same breeding in Petri dishes
150 (diameter: 8.5 cm, height: 2.7 cm) with cowpea seeds infested by *C. maculatus* and a piece of
151 cotton soaked in water. We isolated females at emergence so that they were virgin and we
152 placed them in the presence of a male. We left couples in contact for one to two days so that
153 females could mate and parasitize hosts. We then removed the wasps to allow offspring
154 development (generation 1, G1). At the emergence of G1 offspring (about 15 days after
155 hatching; Jaloux et al., 2004), we mated one son and one daughter from each original mating
156 pair to produce the second generation (G2). We repeated this operation until the 17th generation
157 (G17). Theoretical models estimate that inbred lineages become fixed from G20 in diploid
158 species (Hamilton, 2009). As *E. vuilleti* is a haplodiploid species, fixation process is likely to
159 be more rapid; we therefore assumed that we had generated four different, highly inbred
160 genotypes (referred as genotypes ‘A’, ‘B’, ‘C’ and ‘D’). We then individually placed G17
161 females in Petri dishes with a brother male and supplied them with infested cowpea seeds for 7
162 days, in order to obtain G18 daughters that were (1) familiar (same seed) and related (same
163 genotype), (2) familiar (same seed) and unrelated (different genotypes), (3) unfamiliar
164 (different seeds) and related (same genotype) and (4) unfamiliar (different seeds) and unrelated
165 (different genotypes). To obtain familiar – unrelated females (i.e., coming from the same seed
166 but genetically different), we cut seeds into halves, placed each half in different Petri dishes
167 and allowed G17 mothers from different genotypes to separately parasitize hosts contained in
168 each half of the seeds (Fig. 1). The difficulty in obtaining wasps that emerge synchronously
169 from each half seeds explains the relatively small sample size of contests performed between
170 these unrelated–familiar females (see the ‘experimental procedure’ section).

171 *Females preparation for contests*

172 At the emergence of G18 individuals, we isolated wasps to prevent them from having
173 any competition experience and introduced them separately in Petri dishes supplied with a piece
174 of cotton soaked in water (Fig. 1). During the 3 days preceding behavioural observations, we
175 provided females with one *C. maculatus* pupa, which was replaced daily. To do so, we dissected
176 cowpea seeds to collect pupae. We then individually placed them in standard transparent
177 gelatine capsule (length: 2 cm, diameter: 0.6 cm). These artificial seeds allow for better
178 observation of laid eggs, without altering females' oviposition behaviour (Gauthier & Monge,
179 1999; Jaloux et al., 2004). Moreover, as *E. vuilleti* is a synovigenic species (i.e. they possess a
180 few mature eggs at emergence and egg maturation continues throughout adult life), we added a
181 male to the females' petri dishes for 24h to stimulate oogenesis (Terrasse & Rojas-Rousse,
182 1986). In addition, to facilitate individual identification during contests, we marked them on
183 the dorsal part of their thorax with a dot of bright yellow or bright red acrylic paint. On the day
184 of behavioural observations, we removed the hosts and deprived females of hosts for at least 2h
185 before the start of the contest so that they were more motivated to oviposit. We then observed
186 contests between females fighting for a 4th instar larva of *C. maculatus*.

187

188 *Experimental procedure*

189 At the start of the observation, we simultaneously introduced two parasitoid females
190 into an arena consisting of a plastic block made of three chambers linked by a narrow channel
191 (adapted from Petersen & Hardy, 1996). We placed females in the central chamber already
192 containing a 4th instar larva of *C. maculatus* previously placed in a capsule. Mathiron et al.
193 (2018) showed that females with oviposition pre-experience on pupae were more motivated to
194 gain access to a 4th instar larva. Contestants were of the same age (3 days old), marked with

195 different colours and visually matched for size (*a posteriori* analysis confirmed that contestants
196 did not differ significantly in body mass; Wilcoxon signed-ranks test: $T = 2463$, $N = 92$, $P =$
197 0.14). We set up four different types of contests (Fig. 1), with different combinations of
198 genotypes: (1) females emerged from the same seed that were from the same genotype (i.e.
199 familiar – related, $N = 19$; genotype combinations: ‘AA’, $N = 7$; ‘BB’, $N = 5$; ‘CC’, $N = 2$;
200 ‘DD’, $N = 5$), (2) females emerged from the same seed that were from different genotypes (i.e.
201 familiar – unrelated, $N = 9$; genotype combinations: ‘AD’, $N = 1$; ‘BC’, $N = 2$; ‘BD’, $N = 1$;
202 ‘CD’, $N = 5$), (3) females emerged from different seeds that were from the same genotype (i.e.
203 unfamiliar – related, $N = 25$; genotype combinations: ‘AA’, $N = 9$; ‘BB’, $N = 5$; ‘CC’, $N = 7$;
204 ‘DD’, $N = 4$) and (4) females emerged from different seeds that were from different genotypes
205 (i.e. unrelated – unfamiliar, $N = 39$; genotype combinations: ‘AB’, $N = 6$; ‘AC’, $N = 5$; ‘AD’,
206 $N = 8$; ‘BC’, $N = 6$; ‘BD’, $N = 7$; ‘CD’, $N = 7$). Wasps from the same genotype were full sisters.

207 During all tests, we recorded oviposition and aggressive behaviours displayed by each
208 female. When wasps detect a conspecific (identified by females raising their antennae in the
209 direction of their opponent), they frequently stop their behavioural oviposition sequence
210 (Mohamad et al., 2010). Females can either display defensive behaviour, kicking the opponent
211 with their legs without taking their ovipositor out of the capsule, or they can exhibit a full attack,
212 in which case one parasitoid usually hits her opponent with her head, chases her away from the
213 capsule, or even mounts her. This hitting, chasing, or mounting can lead the loser to quit the
214 central chamber of the arena. We therefore considered only these behaviours, collectively
215 termed as ‘attack’, in the rest of the study. We conducted behavioural observations during 1h
216 or stopped the contests when one female left the central chamber for at least 2 min. We also
217 stopped contests when neither of the females touched the host nor displayed any aggressive
218 interactions for at least 2min. We considered a contest as resolved when a female chased her

219 opponent out of the central chamber, or when only one female displayed oviposition behaviour
220 (i.e. the winning female) during the experiment time.

221 At the end of experiments, we immediately froze the wasps at -20°C . We then weighed
222 them using an electronic balance (Ohaus Discovery® model, accuracy: 0.01mg) and we
223 counted the number of mature eggs in their abdomen (i.e. egg load) by dissecting them. Finally,
224 we noted for each contest the number of eggs laid on the host by the winning females to
225 determine their initial egg load (i.e. at the start of the contest).

226 *Statistical analysis*

227 We analysed our data with the software R (R Development Core Team, 2016; version
228 3.5.0), using $\alpha = 0.05$. Instead of transforming data to fit standard assumptions, we chose to
229 perform parametric analyses in which the assumed distribution of residuals was matched to the
230 data (Wilson & Hardy, 2002; Briffa et al., 2013).

231 We first defined contest aggressiveness as a binary response: 0 = no aggressive
232 behaviour occurred during a replicate and 1 = aggressive behaviours occurred ($N = 92$ contests).
233 We then performed a generalized linear model (GLM) with a binomial error distribution (link
234 function = 'logit') to investigate the influence of absolute difference in initial egg load between
235 contestants, genetic relatedness and familiarity among females on the probability that
236 aggressive behaviours would be exhibited during contest. The model included the three main
237 effects and two-way interactions between variables. We used the same procedure to explore the
238 influence of absolute difference in initial egg load between contestants, genetic relatedness and
239 familiarity among females on the probability of contest resolution. We thus defined contest
240 resolution as a binary response: 0 = the contest was unresolved and 1 = there was a clear winner
241 ($N = 92$ contests).

242 We finally examine the influence of absolute difference in initial egg load between
243 contestants, genetic relatedness and familiarity among females on the total number of attacks

244 displayed by both females during contest. To account for overdispersion, we ran a GLM
245 assuming a quasi-Poisson distribution of residuals (link function = 'log'), considering only
246 aggressive contests (i.e. contests where at least one attack was observed; $N = 76$). The model
247 included main effects and two-way interactions between variables. We also included in the
248 model the logarithm of contest duration as an offset term to account for differences in contest
249 duration.

250 For all analyses, we started with the maximal model and removed non-significant
251 interactions to test the significance of main effects. Likelihood-ratio chi-square and F-statistics
252 were calculated using the 'Anova' function (package *car*), which performs type-II analysis of
253 variance for GLM models, i.e. without *a priori* of the sequence order of explanatory variables
254 (Fox & Weisberg, 2019). Where we found significant interactions between factors, we ran
255 Dunn's post-hoc multiple comparison tests to establish which modalities significantly differed
256 from each other (Dunn, 1961).

257

258 *Ethical Note*

259 We used 372 individuals in this study: 186 females for contests, and 186 males for
260 female insemination. No animal ethics approval was required for this study. Nevertheless, we
261 handled all individuals with care and we kept handling time to an absolute minimum. Acrylic
262 paint used for marking does not cause injury to wasps. Subjects submitted to dissection were
263 chilled in a freezer prior to decapitation.

264

265 **RESULTS**

266

267 *Effect of genotype combination*

268 We first verified that the genotype combination of the contestants did not affect the
269 probability of contests being aggressive (GLM with binomial distribution of residuals: $G_9 =$
270 12.72 , $P = 0.18$), the probability of contest being resolved (GLM with binomial distribution of
271 residuals: $G_9 = 9.91$, $P = 0.36$), or the number of attacks during contest (GLM with quasi-
272 Poisson distribution of residuals: $F_{9,66} = 1.90$, $P = 0.07$). However, this could reflect a lack of
273 statistical power due to small sample sizes.

274

275 *Escalation and resolution of conflicts*

276 The probability of contests being aggressive was significantly influenced by the
277 interaction between genetic relatedness and familiarity of females (GLM with binomial
278 distribution of residuals: estimates \pm S.E. = -17.29 ± 1312.5 , $G_1 = 5.54$, $P = 0.02$, Fig. 2a).
279 Contest escalation was indeed less likely when contestants were familiar and related than when
280 they were familiar and unrelated (post-hoc Dunn's multiple comparison test: $P = 0.02$, Fig. 2a),
281 but no difference was observed between unfamiliar-related and unfamiliar-unrelated females
282 (post-hoc Dunn's multiple comparison test: $P = 0.27$; Fig. 2a). Moreover, no difference was
283 observed between familiar-related and unfamiliar-related females (post-hoc Dunn's multiple
284 comparison test: $P = 0.05$; Fig. 2a), and between unfamiliar-related and unfamiliar-unrelated
285 females (post-hoc Dunn's multiple comparison test: $P = 0.10$; Fig. 2a). The probability of
286 contest being aggressive did not significantly vary with the absolute difference in female egg
287 load (GLM with binomial distribution of residuals: estimates \pm S.E. = -0.115 ± 0.184 , $G_1 = 0.38$,
288 $P = 0.54$). All other interactions were also non-significant.

289 The probability of a contest being clearly resolved varied significantly with familiarity
290 (GLM with binomial distribution of residuals: estimates \pm S.E. = 2.216 ± 0.802 , $G_1 = 11.32$, P
291 < 0.001). Contests between familiar females had a greater chance of showing a clear winner
292 than contests between unfamiliar females (Fig. 2b). However, we found no effect of genetic

293 relatedness (GLM with binomial distribution of residuals: estimates \pm S.E. = -0.332 ± 0.507 ,
294 $G_1 = 0.430$, $P = 0.51$), the absolute difference in female egg load (GLM with binomial
295 distribution of residuals: estimates \pm S.E. = -0.201 ± 0.158 , $G_1 = 1.634$, $P = 0.2$), or any 2-way
296 interactions between variables.

297

298 *Number of attacks during conflicts*

299 The combined total number of attacks displayed by the two wasps during a contest was
300 influenced by the absolute difference in female egg load, with contests being more aggressive
301 when this difference was larger (GLM with quasi-Poisson distribution of residuals: estimates \pm
302 S.E. = 0.229 ± 0.041 , $F_{1,72} = 28.01$, $P < 0.001$; Fig. 3). This total number of attacks did not vary
303 with female genetic relatedness (GLM with quasi-Poisson distribution of residuals: estimates \pm
304 S.E. = -0.059 ± 0.148 , $F_{1,72} = 0.1593$, $P = 0.69$), familiarity (GLM with quasi-Poisson
305 distribution of residuals: estimates \pm S.E. = -0.323 ± 0.170 , $F_{1,72} = 3.795$, $P = 0.06$) or any 2-
306 way interactions between these variables.

307

308 **DISCUSSION**

309 The main goals of our study were: 1) to investigate whether *E. vuilleti* females adjust
310 their aggressive behaviour during contests for host access according to the genetic relatedness
311 with their competitor, and if so, 2) to explore on which components (phenotypic matching
312 and/or familiarity) kin recognition is based. We observed that both genetic relatedness and
313 familiarity influence the probability of conflict being aggressive: contest escalation was the
314 least likely when contestants were both familiar and related. Moreover, familiarity affected
315 conflict resolution, contests being more likely to be resolved when females were familiar. We
316 thus highlighted kin discrimination in *E. vuilleti*, a solitary parasitoid with low dispersion ability

317 (Cortesero et al., 1997). This evidence is all the more important that, in species with limited
318 dispersion, the high risk of competition between kin may weaken the evolution of kin
319 discrimination (West et al., 2001, 2002) and behaviours favouring relatives may not be selected
320 for, as observed in fig wasps (West et al., 2001).

321 Our results first indicate that *E. vuilleti* individuals were able to recognize both
322 genetically related females and familiar females, and then decide whether to engage in an
323 aggressive contest or not. Recognition of relatives in *E. vuilleti* agrees with what was found in
324 the polyembryonic wasp genus *Copidosoma*, in which attack rates by the larval soldier caste
325 was inversely correlated with competitor relatedness (Giron et al., 2004). Moreover, in the
326 gregarious parasitoid wasp, *G. legneri*, contests between adult females over hosts were less
327 aggressive when competitors were more closely related and when females had developed on
328 the same host (i.e., familiar, Lizé et al., 2012). However, several other studies of parasitoid
329 hymenopterans have reported that kin recognition did not affect fights between males (West et
330 al., 2001; Innocent et al., 2011), mate choice (Bourdais & Hance, 2009; Ruf et al., 2010), or sex
331 allocation behaviour (Reece et al., 2004). Recognition of genetically encoded phenotypic cues
332 can operate through two mechanisms: (1) allelic recognition (greenbeard effect), i.e.,
333 recognition of an allele that underlies the expression of a phenotypic trait (Gardner & West,
334 2010) and (2) phenotypic matching, which is based on overall phenotypic similarity between
335 related individuals (Gadagkar, 1985; Mateo, 2004). The first mechanism leads to the
336 recognition of similarity at the locus responsible for expression of the phenotypic trait (Gardner
337 & West, 2010), while the second mechanism leads to relatedness recognition at the genome
338 scale if there is a strong correlation between both genetic and phenotypic similarity (Mateo,
339 2004; Gardner & West, 2010). Because allelic recognition may involve cooperation between
340 unrelated individuals expressing the trait encoded by a given allele, fixation in a population is
341 expected to be rare owing to invasion by cheaters (Gardner & West, 2010). As such, phenotypic

342 matching is more plausible in the current case. Phenotype matching, in which the genotype of
343 individuals is expressed through their phenotype, contrasts to familiarity in which phenotypic
344 cues may derive from genotypes and/or environment (Lizé et al., 2012). Even if phenotypic
345 matching is generally referred to as a different mechanism from familiarity, phenotypic
346 matching may thus be seen as an extension of familiarity (Mateo, 2004). Here, because the
347 probability of a conflict being aggressive was influenced by the interaction between genetic
348 relatedness and familiarity among females, this suggests that phenotypic matching mechanism
349 may be an extension of familiarity in *E. vuilleti* females. In accordance with the study of Lizé
350 et al. (2012) in *G. legneri*, our experiments highlighted that these two components of kin
351 recognition affect contests for hosts in a parasitoid wasp. More strikingly, we showed for the
352 first time that both components can interact to affect intraspecific competition for resources in
353 a solitary insect species.

354 While we also expected the total number of aggressive behaviours displayed during
355 contests to be influenced by both kin recognition components, only the difference in
356 competitors' egg load had a significant effect: fights were less aggressive when the opponents
357 had a similar number of mature eggs. This unexpected result seems in contradiction with theory
358 models predicting that symmetry between individuals leads to more escalated contests of longer
359 duration (Parker, 1974; Maynard Smith & Parker, 1976). Here, one explanation is that the total
360 number of attacks during a contest may have been driven by the behaviour of the contestant
361 with the highest egg load. As egg load is known to influence competitors' subjective value of
362 the resource (Enquist & Leimar, 1987; Stokkebo & Hardy, 2000), the more gravid females (i.e.
363 with more mature eggs in their ovaries) are expected to be more motivated to fight for winning
364 access to the host. Opponent females can match their aggressiveness, resulting in an increase in
365 the total number of aggressive behaviours during conflicts. Additional analyses seem to support
366 this hypothesis: the total number of attacks displayed by the two wasps during contests

367 significantly increased with the egg load of the most gravid contestant (GLM with quasi-
368 Poisson distribution of residuals: estimates \pm S.E. = 0.11 ± 0.04 , $F_{1,74} = 6.20$, $P < 0.02$) and the
369 number of attacks displayed by both females are positively correlated (GLM with quasi-Poisson
370 distribution of residuals: estimates \pm S.E. = 0.04 ± 0.01 , $F_{1,74} = 36.27$, $P < 0.001$). Furthermore,
371 our results add to the evidence that egg load is the major factor driving variation in aggressive
372 behaviours during contests for hosts in *E. vuilleti* (Mohamad et al., 2010, 2012). The underlying
373 mechanism is still unknown, but the involvement of hormones has been suggested (Stokkebo
374 & Hardy, 2000; Goubault & Decuignière, 2012). In particular, juvenile hormone is known to
375 be involved in egg maturation in many insect species (reviewed by Nijhout, 1994) and
376 associated with aggressiveness in different hymenopteran species such as paper wasps (Tibbetts
377 & Huang, 2010) and honeybees (Pearce et al., 2001). Ecdysteroids also play a major role in the
378 regulation of oogenesis in *E. vuilleti* females (Bodin et al., 2009), and may affect female
379 aggressive behaviours. Nevertheless, additional work is required to investigate to what extent
380 hormones may affect female aggressiveness during contest over host.

381 In contrast with Lizé et al. (2012) who found no effect of familiarity or genetic
382 relatedness on the probability of contests being resolved in *G. legneri*, we showed that conflicts
383 between familiar females were more likely to result in a clear winner and loser. In parasitoid
384 hymenopterans, offspring survival, hence females' direct fitness, depends directly on their
385 ability to acquire, parasitize and defend hosts (Petersen & Hardy, 1996). However, exploiting
386 a host, and in particular drilling through the seed to access it, has been shown to be highly
387 energetically costly in this species (Boisseau et al., 2017). Quitting a host, to the benefit of
388 another female, is thus a waste of potential fitness return, but may save energy. Nevertheless,
389 if both contestants are relatives, the increase in inclusive fitness may counterbalance the cost of
390 surrendering the host to a conspecific female, as predicted by kin selection theory (Hamilton,
391 1964). Behavioural mechanisms that allow higher probabilities of contest resolution between

392 familiar females in *E. vuilleti* would therefore be adaptive, as long as emerging from hosts that
393 developed in the same seed is a good predictor of genetic relatedness in this species. In *E.*
394 *vuilleti*, mothers often monopolize a cowpea seed infested by several juvenile *C. maculatus*
395 (Mohamad et al., 2012), and wasps that emerge from the same seed are therefore highly likely
396 to be sisters.

397 Altogether, we observed that *E. vuilleti* females variably rely on genetic relatedness and
398 familiarity cues depending on the contest stage: they first rely on both types of cues when
399 deciding whether to escalate the conflict or not. Then, during escalated contests, aggressiveness
400 varied only with SRV asymmetry between contestants. Finally, contest resolution was based on
401 familiarity cues only. If this may be surprising at first glance, the type of information used by
402 animals for decision-making is classically known to change over contests (see Arnott &
403 Elwood, 2009 for a review of ‘assessment’ models). For example, Elias et al. (2008) showed
404 that males of the jumping spider, *Phidippus clarus*, use multimodal signals during aggressive
405 interactions: the duration of pre-contact phases was based on differences in vibration behaviour
406 between males, while motivation to escalate towards contact phases was mainly based on body
407 size. Moreover, in the mangrove rivulus fish, *Kryptolebias marmoratus*, males use different
408 assessment strategies during conflicts. Fish usually adopt ‘mutual assessment’ to decide
409 whether to escalate the contest, and then change to a ‘self-assessment’ to whether persist the
410 escalation (Hsu et al., 2008). Overall, our results demonstrated that wasps are able to use
411 different cues at each phase of the conflict to make their behavioural decisions.

412 The mechanisms underlying the identification of genetic relatedness and familiarity
413 remain to be explored in *E. vuilleti*. However, *E. vuilleti* females are known to adjust their
414 behaviour based on the detection of conspecific cuticular hydrocarbons (CHC; Darrouzet et al.,
415 2010). Because the wasps, in our study, discriminated competitors of the same genotype and
416 those from the same seed, they may have detected genetically encoded or environmentally

417 influenced differences in the CHC profile of their opponent. Studies in parasitoid hymenoptera
418 suggest that CHC may be characteristic of related individuals (Höller et al., 1991; Howard,
419 2001). Several studies have also measured biochemical convergence between the surface lipids
420 of host plants and cuticular compounds of insect herbivores and their predators or parasitoids
421 (reviewed in Blomquist & Bagnères, 2010). Kühbandner et al. (2012) found, in the pteromalid
422 wasp *Lariophagus distinguendus*, that shifting host during only one generation led to
423 distinguishable CHC profiles among females. Moreover, since familiar *E. vuilleti* females came
424 from the same seed on which the hosts fed, it is possible that females developing on hosts that
425 exploit the same resource might express a similar biochemical profile that allows for
426 recognition of familiar individuals (Ode et al., 1995; Singer, 1998). For example, von Beeren
427 et al. (2011) showed that ant parasites *Malayatelura ponerophila* acquire cuticular
428 hydrocarbons directly from their host *Leptogenys distinguenda*. However, *E. vuilleti* females
429 may have simply acquired seed-specific compounds by friction when emerging out of the seed.
430 Physical contacts can also generate variation in the chemical cuticular hydrocarbon profile:
431 when stroking their abdomens on the nest surface of their host, both parasite species
432 *Sulcopolistes sulcifer* and *Polistes semenowi* acquire host hydrocarbons from the comb
433 (Turillazzi et al., 1990; Zacchi, 1995). Moreover, in social wasps, a hydrocarbon layer similar
434 to that of resident wasps covers nest paper, so that physical contact between parasite cuticle and
435 nest hydrocarbons may favour the transfer of odours between parasites and comb (Lorenzi et
436 al., 1996; Singer, 1998). We call future studies to test these hypotheses and identify the cues
437 involved in the different components of kin recognition.

438

439 *Conclusion*

440 By investigating the effects of both genetic relatedness and common developmental
441 environment on contest behaviour and resolution in *E. vuilleti*, we were able to demonstrate

442 that two kin recognition components (phenotypic matching and familiarity) mediate the
443 occurrence of escalated conflicts and contest resolution in a parasitoid wasp. We showed for
444 the first time that both components can interact to affect intraspecific competition in a solitary
445 insect species. Additionally, in species with limited dispersion like *E. vuilleti* (Cortesero et al.,
446 1997), high competition risk for resources between kin may weaken the evolution of reducing
447 aggressive behaviours toward related conspecifics (West et al., 2001, 2002). Our study therefore
448 shows that kin discrimination during intraspecific competition can also evolve in a species with
449 limited dispersion.

450

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685 **Figure captions**

686 **Figure 1.** Experimental design that allowed us to investigate the role of genetic relatedness and
687 familiarity on female aggressiveness and conflict resolution over host access. Parasitoid females from
688 different genotypes ('A', 'B', 'C' and 'D') mated and then parasitized infested cowpea seeds for 7 days,
689 in order to obtain adult wasps that have emerged from the same seed or not, and that were genetically
690 related or not. After 3 days of preparation, we observed contests between females that were (1) familiar-
691 related, (2) familiar-unrelated, (3) unfamiliar-related and (4) unfamiliar-unrelated, fighting for accessing
692 to a host.

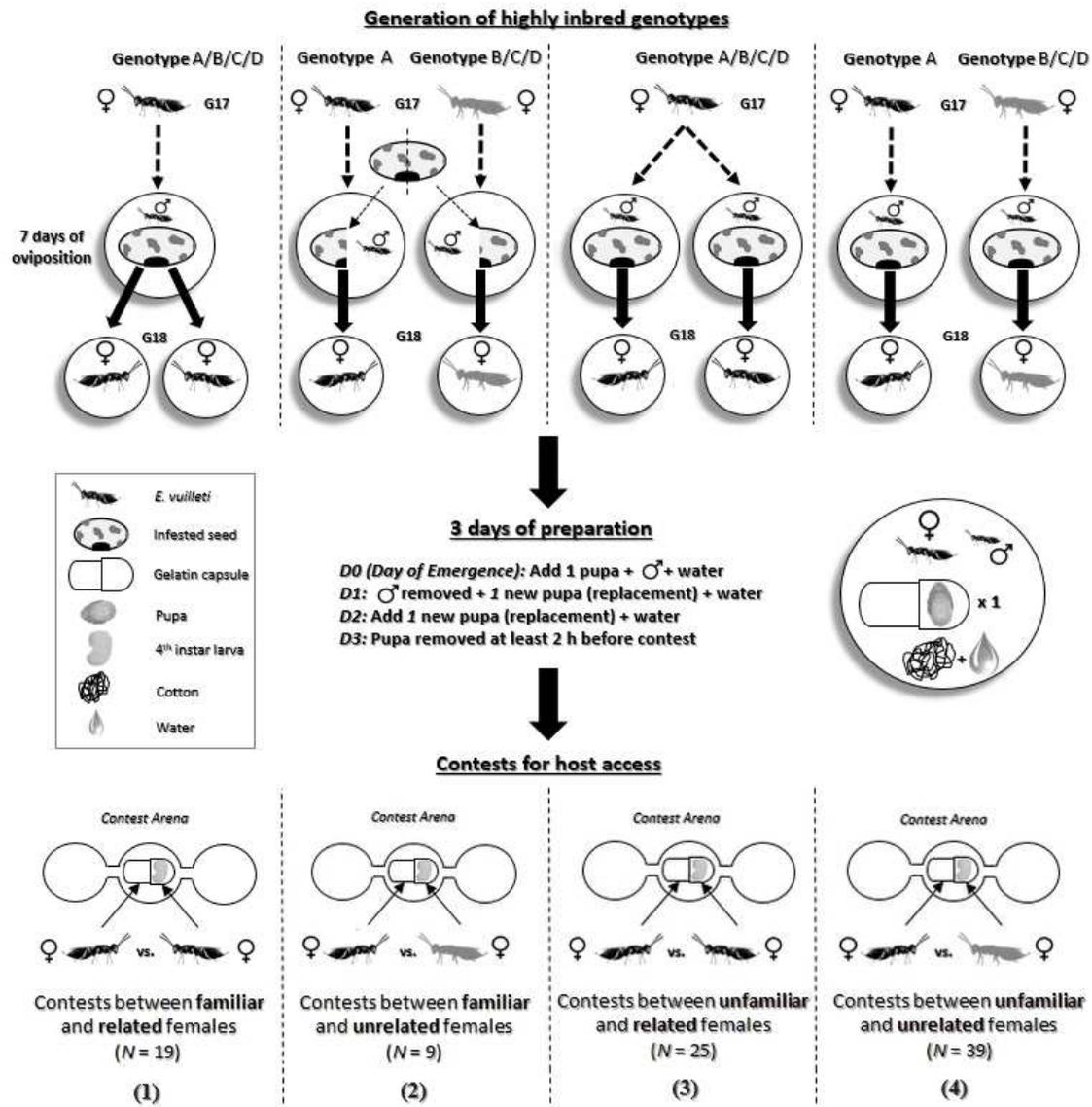
693

694 **Figure 2.** (a) Probability (\pm S.E.M.) of a contest being aggressive when familiar contestants were
695 genetically related (light bars, $N = 19$) or unrelated (dark bars, $N = 9$), or when unfamiliar contestants
696 were genetically related (light bars, $N = 25$) or unrelated (dark bars, $N = 39$). Different letters indicate
697 significant differences based on post-hoc Dunn's multiple comparison test. (b) Probability (\pm S.E.) of
698 a contest being resolved when contestants were familiar (light bars, $N = 28$) or unfamiliar (dark bars, N
699 = 64). ***: $P < 0.001$.

700

701 **Figure 3.** Combined total of number of aggressive behaviours observed during contest according to the
702 absolute difference in initial egg load between females of a same dyad. ***: $P < 0.001$. Statistical
703 analyses accounted for differences in contest duration, and showed that effect of the absolute difference
704 in female egg load was still significant when removing the contest during which we observed 100 attacks
705 (GLM with quasi-Poisson distribution of residuals: estimates \pm S.E. = 0.175 ± 0.049 , $F_{1,71} = 4.87$, $P <$
706 0.01).

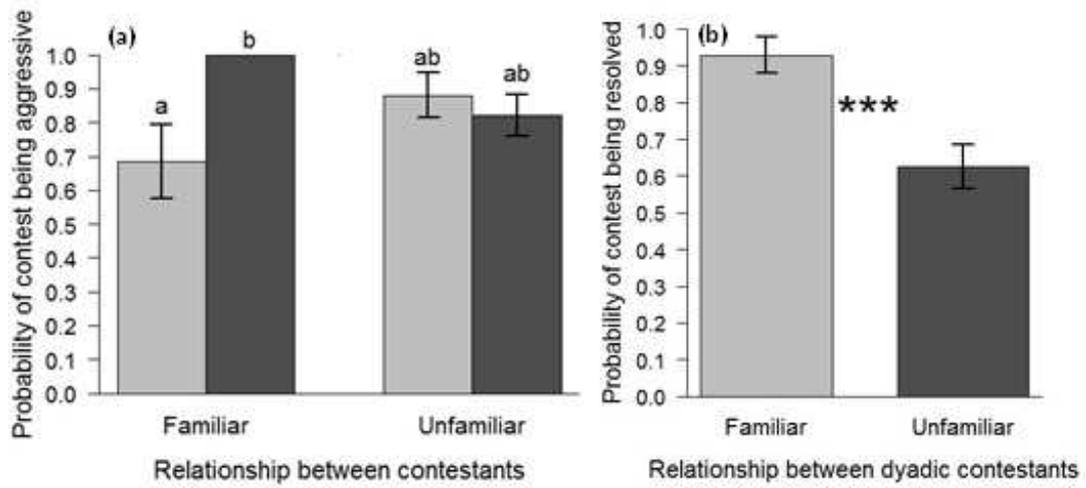
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710 **Figure 1.**

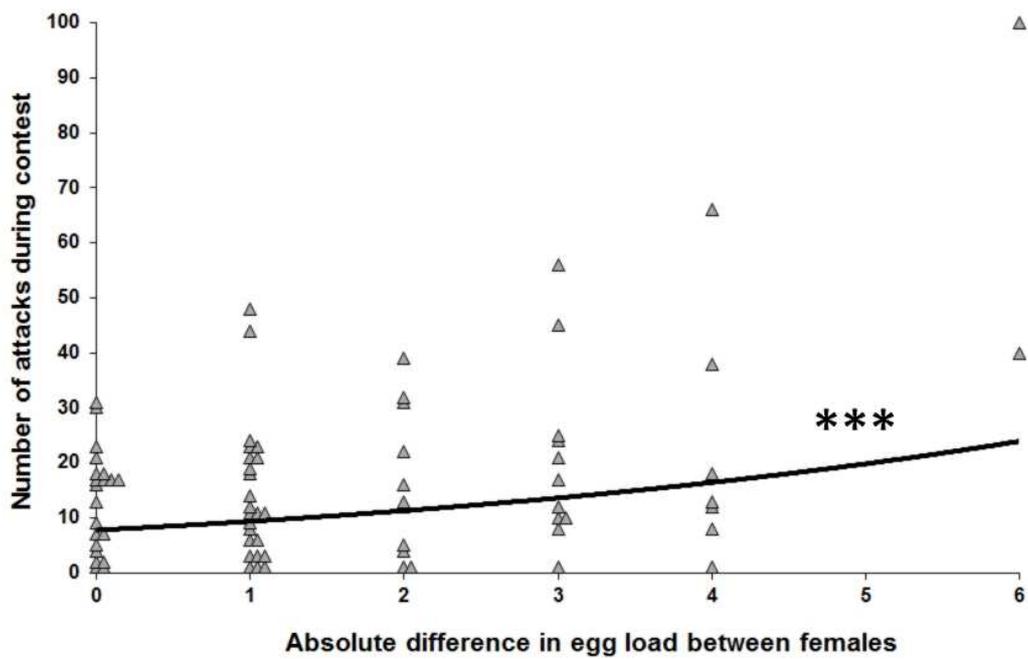
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713 **Figure 2.**

714



715

716 **Figure 3.**