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Cuckoo wasps manipulate host foraging and resting activities through behavioural interactions

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Short title: Host manipulation by social parasite wasp

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27   **ABSTRACT**

28   Parasite-induced alterations in host behaviour have been reported in a large number of

29   taxa. However, some parasites are better than others to exploit the resources offered by their

30   hosts. To date, our understanding of the extent to which some obligate parasites exploit social

31   insect colonies is still limited. In this study, we examined parasite-mediated behavioural

32   alterations of *Polistes biglumis* wasps parasitized by the obligate social parasite *P.*

33   *atrimandibularis* by comparing host female-activity in parasitized and non-parasitized

34   colonies. Host foundresses foraged more and rested less in parasitized than in non-parasitized

35   colonies. Next, we used short-term parasite removal experiments to investigate how social

36   parasites manipulate their hosts. These experiments allowed us to confute the alternative

37   hypothesis that changes in host behaviour were the result of changes in colony needs (usually

38   greater in parasitized than non-parasitized colonies). Parasitized host-foundresses foraged

39   more and their adult female offspring rested less when social parasites were on the nest rather

40   than after their removal. *P. atrimandibularis* may use behavioural interactions (such as

41   antennation and trophallaxis) to manipulate host activities, rather than visual, acoustic, or

42   chemical signals as other parasites do.

43

44   Key words: *Polistes*, social insects, host-parasite system, behavioural manipulation,

45   proximate mechanism, brood parasitism, dominance-subordinance interactions

46

INTRODUCTION

47

48   In interactions between parasites and their hosts, parasites are selected for their ability to

49   manipulate their hosts and enhance their opportunities for reproduction, transmission, or host

50   encounter (Combes 1991; Poulin and Thomas 1999; Combes 2001). Examples of changes in

51   host phenotype induced by endoparasites have been documented in a large number of taxa,

52   ranging from viruses to protozoan, and from flatworms to helminths (reviewed in Schmid-

53   Hempel 1998; Thomas et al. 2005a; Lefèvre et al. 2008). For instance, a nematode changes

54   the colour and behaviour of infected Turtle ants (Cephalotes) which are its intermediate hosts

55   leading to trophic transmission into birds, the final host (Yanoviak et al. 2008). Similarly,

56   another nematode induces a bioluminescent coloration in its host insect larva, causing avian

57   predators to avoid affected larvae as prey and thus preventing itself and its host from being

58   eaten (Fenton et al. 2011). The alteration of host behaviour by parasites is a strategy of host

59   manipulation that is especially impressive and is well documented in viruses, bacteria,

60   protozoan, arthropods, and birds (e.g., Soler et al. 1995a; Soler et al. 1995b; Berdoy et al.

61   2000; Moore 2002; Klein 2003; Ponton et al. 2006; Rogers and Bates 2007). An obligate

62   endoparasitic strepsipteran induces its host wasps, for example, to desert their colonies and

63   aggregate outside, enhancing parasite opportunity for mating (Hughes et al. 2004).

64   Ectoparasites manipulate and alter host phenotypes by exploiting the chemistry,

65   physiology, morphology, and behaviour of their hosts (Nash and Boomsma 2008). For

66   example, cowbird young use begging calls to elicit enhanced food provisioning by their host

67   parents (Gloag and Kacelnik 2013). The larvae of a parasitic wasp induce their spider hosts to

68   build a modified web for their own protection (Eberhard 2010). Social parasites can limit

69   attacks by their social insect hosts using chemical weapons (e.g., D'Ettorre et al. 2000; Mori

70   et al. 2000), and/or intercepting the host communication-code, which facilitates host nest

71   invasion and integration (Lenoir et al. 2001; Thomas et al. 2005b; Nash and Boomsma 2008;

4

72 Bagnères and Lorenzi 2010). Host manipulation by social parasites is impressive because it

73 involves entire colonies rather than one or a few hosts (Hughes 2008).

74   Among the primitively eusocial *Polistes* wasps (Hymenoptera, Vespidae, Polistinae), there

75 are three species of obligate and permanent social parasites: *P. sulcifer*, *P. semenowi*, and *P.*

76 *atrimandibularis*. As obligate parasites, they depend entirely on host social structures, lack

77 the worker caste, and use their host workforce to rear their own brood, which is composed

78 exclusively of reproductives (Cervo and Dani 1996). *Polistes* parasites have evolved

79 morphological and chemical adaptations that facilitate invasion and integration in host

80 colonies (Cervo and Dani 1996; Cervo 2006; Lorenzi 2006; Bagnères and Lorenzi 2010). To

81 date, no evidence exists of a pheromonal control in *Polistes* wasps and dominance-

82 subordinance interactions are almost entirely behaviourally mediated (Pardi 1948; West-

83 Eberhard 1969; Reeve 1991). Similarly, behavioural dominance acts seem to be the only way

84 social parasites establish relationships with their hosts, as neither pheromonal nor acoustic

85 communication channels play any role (Cervo, 2006). *Polistes* social parasites may therefore

86 manipulate and alter host activities through behavioural interactions. A similar strategy is

87 used by *P. fuscatus* queens to regulate the activity of their own workers (e.g. foraging effort)

88 and monopolize egg-laying (Reeve and Gamboa 1983, 1987), although this strategy is not

89 widespread (Jha et al. 2006).

90   In this study, we used the *P. biglumis* - *P. atrimandibularis* host-parasite system to assess

91 whether and how social parasites controlled and altered their host behaviour. We

92 hypothesised that parasites used behavioural interactions to induce host females to forage

93 intensively to the advantage of parasite brood. To test this hypothesis, we compared host

94 behaviour in parasitized and non-parasitized colonies and ran short-term parasite removal

95 experiments. Our experiments simulated a naturally-occurring phenomenon: each day

96 parasites leave their nests several times to forage (Cervo 2006) and sometimes they do not

97   return, possibly due to fatalities (personal observations). Removal experiments have been

98   routinely used in the study of social regulation in *Polistes* colonies (e.g., Reeve and Gamboa

99   1987; Tibbetts and Huang 2010).

100

MATERIALS AND METHODS

The Species and their Interaction

Polistes biglumis wasps live in mountain zones (1600-2350 m a.s.l.) in Southern Europe. The severe climatic conditions reduce the length of the nesting period to 3.5-4 months (from late May to September). Colonies are always strictly founded by single foundresses (solitary foundation) and are small, i.e., they produce less than 30 individuals throughout the nesting season (Lorenzi and Turillazzi 1986). In the studied population, the single foundress is the most active female even after brood emergence, as most of the emerging females are actually reproductive females (Fucini et al. 2009; Lorenzi and Thompson 2011). Although *P. biglumis* wasps have efficient recognition systems (Lorenzi et al. 1997; Lorenzi and Filippone 2000), their nests are targeted by cleptoparasites (Uboni and Lorenzi 2013), by conspecific social parasites (Lorenzi and Cervo 1995), and by obligate social parasites (*P. atrimandibularis*) (Cervo et al. 1990b). Obligate social parasites overcome host detection by possessing few recognition cues and gradually matching those of their hosts, so that hosts accept their parasites as nestmates (Bagnères et al. 1996; Lorenzi et al. 1999; Lorenzi and Bagnères 2002; Lorenzi 2003; Uboni et al. 2012). Obligate social parasites may invade *P. biglumis* colonies about a month after their foundation, when the foundress is still the only adult wasp in the colony (pre-emergence phase; Cervo et al. 1990a). Since parasites peacefully invade host colonies and do not kill or harm host foundresses, parasite and host foundress cohabit in the parasitized nest (Lorenzi et al. 1992). When parasites enter host colonies, they destroy host eggs and young larvae (but spare old larvae and pupae of the host), suppress host foundress reproductive capacity, and begin laying their own eggs (Cervo et al. 1990a; Cervo and Lorenzi 1996). Parasitized colonies will then produce first host brood and later parasite brood.

Study Site and Field Methods

127   Behavioural observations and experiments were conducted in the field, near Montgenèvre

128   (44°55'N, 6°43'E, Hautes Alpes, France, 1850 m a.s.l.), in a typical Alpine environment with

129   *Pinus silvestris*, *Larix decidua*, and wide meadows. We analysed a *P. biglumis* population

130   where *P. atrimandibularis* has been recorded since 1958. Up to 20-40 % of founded nests

131   may be parasitized annually (Lorenzi and Thompson 2011).

132   We found 98 *P. biglumis* colonies in different summers (1997, 1998, 2001, 2002, 2006,

133   and 2007). We individually marked the adult members of each colony on the thorax (i.e., the

134   host foundress, its adult female offspring, and the parasite female, where present) with unique

135   colour combinations of enamel paint. In few cases, colonies were discovered later in the

136   season than others. In this case, foundresses were identified by their worn wings. The tips of

137   wasp wings become more and more worn as the wasp performs its daily activities. Thus, the

138   longer a wasp has emerged as an adult, the more worn its wings are.

139

140   *Behavioural Observations in Parasitized vs. Non-parasitized Colonies*

141   We conducted behavioural observations on 33 parasitized and 35 non-parasitized colonies

142   (hereafter, untreated colonies) during both the host pre- and post-emergence phases, for a

143   total of 368 h, by using a Canon MV960 camcorder, placed at approximately 20 cm from the

144   nest (two observations per colony, each about 2 hours long, see Table 1 for details).

145   Behavioural observations were performed at least 10 days after the period when parasites

146   invade host nests, so that in parasitized colonies all larvae were parasite brood. Behavioural

147   observations were performed on sunny days, between 10:00-16:00 h local time, after

148   checking the number of brood in the nest (eggs, larvae and pupae). For data elaboration, we

149   calculated the amount of time spent by the host females motionless or grooming (hereafter,

150   resting). We also recorded departure and return times from/to nests to quantify the time spent

151 foraging. These behaviours (staying motionless, grooming, leaving the nest, returning to the

152 nest) are easily scored and clearly distinguishable from any other behaviour. Behavioural data

153 were analysed as proportion of observation time.

154

155 *Parasite Removal Experiment*

156 Colony productivity is typically higher in parasitized than in non-parasitized colonies

157 (mean number of larvae ± SD: 36 ± 11 vs. 24 ± 13; Lorenzi et al. 1992), and therefore the

158 number of larvae is larger in parasitized than in non-parasitized colonies. Thus, the two types

159 of colonies presumably differ in the amount of food required to feed larvae (i.e, in colony

160 needs). Additionally, in parasitized colonies, enhanced foraging activity of host foundresses

161 and their adult offspring might be caused by begging by parasite larvae, rather than by

162 manipulation of host behaviour by adult parasite. To test whether differences in host

163 behaviours were associated to the presence of adult parasites on the nest or rather to its brood

164 begging hosts for food, we performed short-term parasite removal experiments on another

165 group of parasitized colonies (hereafter called treated colonies, n = 30). Short-term removal

166 experiments have been previously used to test how queens regulate their worker activities in

167 *Polistes* colonies (Reeve and Gamboa 1987). Experiments were conducted in the field and

168 were not blind because of visible morphological differences between species. However, wasp

169 behaviours were sufficiently clear to avoid subjective interpretation by the observer. All

170 observations were performed by the same observer (S.F.) who recorded the time when the

171 behaviours of interest begun or stopped (no video recording).

172 We performed 4-hour behavioural observations on these colonies, as follows. Before

173 parasite removal, we performed a 2-hour behavioural observation (control observation) on

174 each colony, for a total of 58 h of observation. Then, each parasite was removed from its

175 colony and kept in a fresh box with water and honey for 4 hours. A 2-hour behavioural

9

176   observation (experimental observation) was performed during the 3rd and 4th h after parasite

177   removal, for a total of 54 h. Therefore each experiment lasted 6 hours. Few behavioural

178   observations were slightly shorter than two hours, due to changes in weather conditions that

179   interrupted wasp activity, leading to a total of 112 hours of observation. The time period of

180   parasite removal was limited to 4 hours to limit potential damages to these rare social

181   parasites. At the end of the experiment, each parasite was re-introduced into its host colony

182   and both parasites and hosts soon resumed their typical activities. Therefore, no parasite was

183   harmed during the parasite-removal experiments. The number of larvae did not vary within

184   the 2-hour intervals before and after parasite removal. Eleven colonies used in this

185   experiment were in the pre-emergence phase (n = 6 in 2001, n = 5 in 2002), while 19 were in

186   the post-emergence phase (n = 11 in 2001, n = 8 in 2002). In 10 pre-emergence and 10 post-

187   emergence colonies, the pre-parasite removal observation was conducted between 10:00 and

188   12:00, while the post-parasite removal observation was conducted between 14:00 and 16:00.

189   As a control for effect of time of the day on wasp behaviour, we conducted pre-parasite

190   removal observations on 1 pre-emergence and 9 post-emergence colonies between 12:00 and

191   14:00 and paired these observations to post-parasite removal observations conducted at the

192   same time the following day.

193   During these observations, we recorded the amount of time spent by the host females

194   motionless or grooming (i.e., resting) and departure and return times from/to nests to quantify

195   the time spent foraging, as described above for the behavioural observations on untreated

196   colonies. Before parasite removal, the observer also noted the behavioural interactions

197   between parasites and their hosts, and whether the host or the parasite started them. All

198   interactions were analysed as rates (number of acts/h) and classified as either strongly

199   aggressive (darts, dominance-subordinance interactions, and sting attempts), or non-

200   aggressive (antennation and trophallaxis). All these behaviours are clear-cut and easily

201   identified in the small colonies of this species.

202

203   *Statistical Analyses*

204   To avoid pseudoreplications, each colony supplied a single datum. Differences in host

205   behaviour between parasitized and non-parasitized colonies were analysed using Generalized

206   Linear Mixed Models for binomially distributed data and logit link function (e.g., time

207   foraging/total observation time). Wasp activities may be affected by the number of larvae, as

208   well as by the time of the day and the day of season. Therefore, we included in the models the

209   number of larvae, time of the day and day of the season as covariates. We also included year

210   as a random factor. We used Paired sample t-test in pairwise comparisons (i.e., to analyse

211   data from parasite removal experiments). Statistical analyses were performed using SPSS

212   21.0 statistical package (SPSS Inc, Chicago, IL).

213

214   **RESULTS**

215   *Behavioural Observations in Parasitized vs. Non-parasitized Colonies*

216   *Foundress behaviour*

217   Foundresses foraged significantly more and rested significantly less in parasitized than in

218   non-parasitized colonies and in both the pre- and post-emergence phase (Fig. 1a, b). The

219   variations in foundress foraging and resting between parasitized and non-parasitized colonies

220   were larger in the pre-emergence than in the post-emergence phase (as shown by the

221   significant interaction term colony status * phase) (GLMM on foraging, colony status: $F_{1,76}$ =

222   1284.845, P < 0.0001; pre/post-emergence phase: $F_{1,76}$ = 1524.528, P < 0.0001; colony status

223   * phase: $F_{1,76}$ = 1206.252, P < 0.0001; number of larvae: $F_{1,76}$ = 3349.922, P < 0.0001; time

224   of the day: $F_{1,76}$ = 3767.891, P < 0.0001; day of the season: $F_{1,76}$ = 1033.981, P < 0.0001;

225   random factor year: Z = 1.224, P = 0.221) (GLMM on resting, colony status: $F_{1,76}$ =

226   1350.196, P < 0.0001; pre/post-emergence phase: $F_{1,76}$ = 11147.573, P < 0.0001; colony

227   status * phase: $F_{1,76}$ = 2551.289, P < 0.0001; number of larvae: $F_{1,76}$ = 3497.269, P < 0.0001;

228   time of the day: $F_{1,76}$ = 3497.269, P < 0.0001; day of the season: $F_{1,76}$ = 1307.007, P <

229   0.0001; random factor year: Z = 1.224, P = 0.221) (Fig. 1a, b).

230

231   *Worker behaviour*

232   Similarly to their foundresses, workers foraged significantly more and rested significantly

233   less in parasitized than in non-parasitized colonies (Fig. 1a, b) (GLMM on foraging, colony

234   status: $F_{1,43}$ = 3574.681, P < 0.0001; number of larvae: $F_{1,43}$ = 7834.205, P < 0.0001; time

235   of the day: $F_{1,43}$ = 1323.201, P < 0.0001; day of the season: $F_{1,43}$ = 15189.188, P < 0.0001;

236   random factor year: Z = 1.222, P = 0.222) (GLMM on resting, colony status: $F_{1,37}$ =

237   6568.117, P < 0.0001; number of larvae: $F_{1,37}$ = 1830.293, P < 0.0001; time of the day:

238     $F_{1,37} = 1301.789$, $P < 0.0001$; day of the season: $F_{1,37} = 49999.297$, $P < 0.0001$; random

239   factor year: $Z = 1.225$, $P = 0.221$) (Fig. c).

240

241   *Parasite Removal Experiment*

242   *The pre-emergence phase*

243   During the pre-emergence phase, host foundresses foraged significantly less and rested

244   significantly more after parasite removal (compared to before parasite removal; Paired

245   sample t-test, foraging: $t = 4.071$, $df = 10$, $P = 0.002$; resting: $t = -3.922$, $df = 10$, $P = 0.003$;

246   Fig. 2a).

247

248   *The post-emergence phase*

249   The same variations in host foundress behaviour were observed during the post-emergence

250   phase, as host foundresses foraged less and rested more after parasite removal (foraging: $t =$

251   4.763, $df = 18$, $P < 0.0005$; resting $t = -3.771$, $df = 18$, $P = 0.001$; Fig. 2b).

252   Adult host female offspring responded to parasite removal by significantly increasing the

253   time spent resting ($t = -2.100$, $df = 18$, $P = 0.050$), but did not change the time spent foraging

254   ($t = 1.435$, $df = 18$, $P = 0.168$; Fig. 2c).

255

256   *Behavioural interactions*

257   Before parasite removal, parasites started non-aggressive interactions towards host

258   foundresses significantly more often than host foundresses did towards their parasites, both in

259   the pre- and the post-emergence phase (pre-emergence: $t = -4.935$, $df = 10$, $P = 0.001$; post-

260   emergence: $t = -4.843$, $df = 18$, $P < 0.0005$; Fig. 3a). Strongly-aggressive interactions were

261   very rare from both parts and were started by parasites as often as by host foundresses (pre-

262   emergence: $t = -0.279$, $P = 0.784$; post-emergence: $t = -1.531$, $P = 0.157$; Fig. 3b).

13

263   Parasites interacted significantly more often with adult host offspring than host

264   foundresses did (non-aggressive interactions: N = 19, t = -3.966, df = 18, P = 0.001; strongly-

265   aggressive interactions: N = 19, t = -2.162, df = 18, P = 0.044; Fig. 3c).

266

DISCUSSION

This field study provides evidence that *P. atrimandibularis* obligate social parasites deeply

altered the time that hosts allotted to different activities on their colony. *P. atrimandibularis*

parasites possibly used behavioural interactions to manipulate host behaviour.

The observations in untreated colonies indicated that there were behavioural changes in

both host foundresses and their adult offspring. Indeed, foundresses in parasitized colonies

foraged more and rested less than those in non-parasitized colonies in both the pre-emergence

and the post-emergence phase. Similarly, host offspring foraged more and rested less in

parasitized than in non-parasitized colonies. These results were obtained controlling for the

number of larvae in the nest, the time of the day, and the day of the season. In all cases the

analyses showed that these variables had a significant impact on host foraging and resting,

but nest status (i.e., whether the nest was parasitized or not) was a highly significant predictor

of host behaviour. Therefore, these results suggest that either adult parasites or parasite larvae

(e.g., through "begging" signals; Cervo et al. 2004) directly stimulated an enhanced activity

level in the hosts of parasitized colonies.

Parasite removal experiments indicated that parasite presence caused hosts to increase

their activity levels and highlighted that parasites manipulated their host behaviour during the

whole nesting season. We found that host foundresses and host offspring foraged less and

rested more after parasite removal, even though the number of larvae in the nests did not

change during the experiment. Therefore, we found no support for the hypothesis that hosts

foraged more in parasitized colonies because they were stimulated by signals emitted by

parasite larvae (e.g., "begging" signals; Cervo et al. 2004). Moreover, we would usually

expect to find increased foraging activity during warmer hours (i.e., during 20 out of 30 post-

parasite removal observations), due to the positive effect of air temperature on *Polistes*

foraging activity (Fucini et al. 2014). Instead, we found the opposite pattern (i.e., host

15

292 foundresses and host offspring foraged less and rested more after parasite removal). This

293 finding gives further support to our hypothesis that parasites induce their hosts to forage more

294 and rest less.

295 *P. atrimandibularis* parasites contribute to prey foraging as they plunder larvae from host

296 nests other than the one they live in (Cervo et al. 1990b; personal observations). Therefore,

297 removing parasites actually reduced the amount of food brought to the colonies, and should

298 have increased colony needs and consequently promoted an increase in host activity. This

299 was not the case in our observations, suggesting that parasites actively induced host

300 foundresses to forage more and adult host offspring to rest less on the nests. Similarly,

301 removing *P. fuscatus* queens from their colonies induced a decrease in worker activity (Reeve

302 and Gamboa 1987).

303 Foraging reduces the opportunity for direct reproduction in social wasps more than any

304 other colony task because of the high energetic cost and mortality risk (Markiewicz and

305 O'Donnell 2001). Indeed, foundresses may die prematurely in parasitized nests due to

306 overworking (Lorenzi et al. 1992). Therefore, *P. atrimandibularis* parasites attain great

307 benefits by overexploiting their hosts, at enormous costs for their hosts.

308 Additionally, the results of parasite removal experiments suggested that parasites may

309 stimulate and manipulate host female behaviour via behavioural interactions, i.e. mainly via

310 non-aggressive interactions. Parasites frequently interacted with their hosts and were also the

311 main initiators of interactions with host foundresses and their adult offspring. In *Polistes*

312 wasps, dominant individuals mediate and regulate the activity of subordinate females by

313 means of behavioural interferences, without relying on pheromones (Pardi 1948; West-

314 Eberhard 1969; Reeve 1991). *P. atrimandibularis* may use similar means to control the

315 activity levels of its hosts. Our results support the hypothesis that parasites act as dominant

316 individuals on host nests.

16

317   Examples of behavioural manipulations of hosts by social parasites reported in the

318   literature involve mainly the use of signals produced by parasites and perceived by the hosts,

319   such as visual, acoustic, or chemical signals (Soler et al. 1995a; Kilner et al. 1999; Lenoir et

320   al. 2001; Cervo et al. 2004; Bagnères and Lorenzi 2010). In those cases, parasites manipulate

321   their hosts intercepting specific sensory channels (Combes 2001). After invasion, *P.*

322   *atrimandibularis* acquires the chemical signature of its host nest (Bagnères et al. 1996).

323   Therefore, there is no support for the hypothesis that this parasite uses cuticular chemistry to

324   influence host behaviour. Most probably, behavioural interactions initiated by parasites are

325   relatively more important than chemical cues as ways for host manipulation. However,

326   additional studies will be necessary to support this hypothesis.

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472

473   Table 1. Sample size of the behavioural observations conducted in the field on untreated
474   colonies.

	Pre-emergence phase		Post-emergence phase	
	2006	**2007**	**1997**	**1998**
Parasitized colonies	17	2	7	7
Non-parasitized colonies	12	9	3	11

475

476　　　**Fig. 1** Mean percentage + SE of the observation time spent foraging and resting in

477　　parasitized and non-parasitized colonies by a) host foundresses during the pre-emergence

478　　phase, b) host foundresses during the post-emergence phase, and c) by host female offspring.

479

480　　　**Fig. 2** Mean percentage + SE of the observation time spent foraging and resting before and

481　　after parasite removal by a) host foundresses during the pre-emergence phase, b) host

482　　foundresses during the post-emergence phase, and c) by host female offspring.

483

484　　　**Fig. 3** Mean rate of interactions (mean number of acts / h) + SE between parasites and host

485　　foundresses a) in the pre-emergence phase, b) in the post-emergence phase, and c) mean rate

486　　of interactions initiated by parasites and foundresses towards host offspring. Data were

487　　collected on treated colonies before parasite removal.