

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27

**Female body size, weight and fat storage rather than nestmateship determine male attraction in the invasive yellow-legged hornet *Vespa velutina nigrithorax***

CAPPA F.<sup>1,\*</sup>, CINI A.<sup>1,2</sup>, PEPICIELLO I.<sup>1</sup>, PETROCELLI I.<sup>1</sup> and CERVO R.<sup>1</sup>

<sup>1</sup>*Dipartimento di Biologia, Università di Firenze, Via Madonna del Piano, 50019, Firenze, Italy.*

<sup>2</sup>*Centre for Biodiversity and Environment Research, University College London, Gower Street, London WC1E 6BT, UK*

In the early stage of the invasion process, alien species may face costs linked to pioneer effect due to genetic bottleneck, drift and the consequential inbreeding depression. Thus, introduced species that show an immediate exponential growth in their invasive population should have some mechanisms to reduce such costs minimizing the chance of inbreeding or allowing them to cope with them. The yellow-legged hornet *Vespa velutina nigrithorax* has been spotted in France in 2004; since then, the species has been invading Europe with a relentless pace. In their native range, males and reproductive females of a Chinese non-invasive sub-species of *V. velutina* seem to leave their nests to search for unrelated partners. However, previous studies showed a low genetic diversity and a high rate of diploid males in colonies of the invasive population, suggesting that mating could occur inside nests, where males should be able to discriminate between reproductive gynes and sterile workers. Here, we used laboratory behavioural assays to investigate the mating preferences of yellow-legged hornet males from the recent invasive population in Italy. We assessed the importance of nestmateship and female morpho-physiological traits, likely indicators of caste, in determining male attraction towards potential partners. Our results demonstrate that males are more attracted to bigger females with more abundant fat storage, good indicators of female reproductive caste in wasps, regardless of nestmateship. Our findings represent a first step in understanding the reproductive biology of *V. velutina nigrithorax* in its invasive range, providing a framework for future research in the field to prevent or reduce the species expansion.

28 KEY WORDS: invasive alien species, nestmate recognition, reproductive biology, Asian hornet.

29

30 \*Corresponding author: Federico Cappa, Dipartimento di Biologia, Università di Firenze, Via  
31 Madonna del Piano, 50019, Firenze, Italy (Email: [federico.cappa@unifi.it](mailto:federico.cappa@unifi.it)).

32

33 Running head: Male reproductive attraction in Asian hornets

34

35 Highlights

- 36 - We investigated mating preferences of invasive hornet males under laboratory standard conditions
- 37 - Males attraction was influenced by female caste, with more attraction toward reproductive females
- 38 - Unexpectedly, males were not more attracted by non-nestmate females than nestmate ones.
- 39 - Our results might explain the high level of inbreeding found in invasive populations of *V. velutina*

40

41

## INTRODUCTION

42 Invasive species and their management have represented a major issue in recent years  
43 (Chapin et al. 2000; Lowe et al. 2000; Mooney & Cleland 2001). As the volume of global trade has  
44 expanded enormously in the last decades, so has the risk of invasive alien species (IAS) reaching  
45 new regions with dramatic consequences in terms of biodiversity, ecosystem services' loss and  
46 economic or public health issues (Lonsdale 1999; Mooney & Cleland 2001; Levine & D'antonio  
47 2003; Lockwood et al. 2005; Pejchar & Mooney 2009). Thus, it is important to understand the  
48 processes that promote and accompany the spread of introduced IAS to find sustainable  
49 management solutions (Sakai et al. 2001; Lee 2002; Allendorf & Lundquist 2003; Mehta et al.  
50 2007; Pyšek & Richardson 2010).

51 The invasion process can start with either a single introduction or multiple introductions  
52 over time of a scant number of individuals able to endure sudden habitat transitions (Lee 2002).

53 Because of their low number, introduced individuals might likely face reproduction via inbreeding,  
54 which could strongly affect their fitness by allowing for the expression of deleterious recessive  
55 alleles and decreasing allelic diversity within the gene pool in the emerging population (i.e.  
56 inbreeding depression) (Fonseca et al. 2000; Tsutsui et al. 2000; Rasner et al. 2004). Thus, in order  
57 to be successful in their invasion, IAS should present effective mechanisms to either avoid or  
58 minimize inbreeding, or be able to cope with its costs (Sakai et al. 2001; Lee 2002; Frakham 2005).  
59 Unfortunately, the mating behaviour in IAS pioneer populations is relatively understudied, as  
60 research often focuses on other aspects of the invasion process such as population genetics related  
61 to the rapid spread or IAS impact in the invaded range (Sakai et al. 2001; Lee 2002; Davidson et al.  
62 2011).

63 Eusocial Hymenoptera are especially successful IAS because of their reproductive strategies  
64 and social habit (Moller 1996; Hingston & McQuillan 1999; Chapman & Bourke 2001; Holway et  
65 al. 2002; Paini & Dale Roberts 2005; Inoue et al. 2008; Beggs et al. 2011). A single mated queen  
66 can found a new colony and its social organization, with a considerable number of sterile workers  
67 engaged in rearing and defending the immature brood and the production of a high number of  
68 reproductive individuals each season, can favour the rapid establishment of a new population  
69 (Moller 1996; Holway et al. 2002; Beggs et al. 2011).

70 However, small colonizing populations may face difficulties due to the production of  
71 genetic ‘misfits’ known as diploid males (Whiting 1933; Liebert et al. 2010), which pose fitness  
72 costs to the colony because they consume resources without contributing to colony productivity,  
73 and are functionally sterile (Liebert et al. 2004). Diploid males are produced because of the  
74 Hymenopteran complementary sex determination (CSD) mechanism, which requires heterozygosity  
75 at sex-determining loci to produce normal diploid females (Crozier 1971; van Wilgenburg et al.  
76 2006). Homozygosity at these loci results in diploid males instead of females (Whiting 1933, 1943).  
77 Individuals in an incipient invasive population may at first have limited or no access to unrelated  
78 partners and the fitness costs of not mating is likely to be greater than producing a reduced worker

79 brood alongside diploid males (Liebert et al. 2010). Thus, for individuals may be advantageous to  
80 have a low acceptance threshold for inbreeding avoidance when choosing their potential mates  
81 (Starks et al. 1998a, 1998b; Liebert et al. 2010) and this characteristic could contribute to the  
82 success of invasive species.

83         Among the IAS which have recently invaded Europe, one causing major concern is  
84 undoubtedly the Asian yellow-legged hornet, *Vespa velutina nigrithorax* (Monceau et al. 2014).  
85 The alien hornet was accidentally introduced into Europe from China probably in a single event  
86 (Arca 2012), and was first observed in 2004 in South-West France (Rortais et al. 2010; Monceau et  
87 al. 2014). The species poses serious concerns both from the ecological and economic point of view,  
88 with its impact on both wild and managed pollinators populations as well as on native competitive  
89 species (Shah & Shah 1991; Tan et al. 2007; Gallai et al. 2009; Villemant et al. 2011; Cini et al.  
90 2018), and for public health, since the hornet is adapted to nest in urban environments (Choi et al.  
91 2012) and its sting can induce severe allergic reactions, resulting in organ failure and death (Choi et  
92 al. 2012; Liu et al. 2015). Thus, it is of crucial importance to know the biology of the invader in  
93 order to adopt the most successful strategies for managing it with the lowest impact on native  
94 species communities (Mehta et al. 2007; Beggs et al. 2011).

95         To date, however, scarce information is available on the reproductive behaviour of *V.*  
96 *velutina nigrithorax* both in its native and invasive range (Monceau et al. 2014). In the invasive  
97 populations it has been hypothesized that mating could occur inside the nest because of the high  
98 rate of diploid males and inbreeding in the European population (Monceau et al. 2014; Darrouzet et  
99 al. 2015). Moreover, aggregations of courting males, observed in other Vespinae species (Matsuura  
100 & Yamane 1990; Ross & Carpenter 1991; Wen et al. 2017) have never been described in the  
101 invasive population of *V. velutina nigrithorax* (Monceau et al. 2014; Darrouzet et al. 2015),  
102 although males of a different Asian subspecies of *V. velutina* distributed in Yunnan are attracted in  
103 the field by a volatile sex pheromone produced by the sixth intersegmental sternal glands of virgin  
104 gynes (i. e. reproductive females) (Wen et al. 2017).

105 Here, we use behavioural bioassays in the laboratory to investigate the first phase of the  
106 reproductive behaviour of the invasive *V. velutina nigrithorax*, by focusing on the male attraction  
107 towards females to assess the potential effect of nestmateship and physiological features (weight,  
108 body size, fat stores) linked to caste in the male search for a potential partner. Reproductive gynes  
109 start to emerge in Autumn, after the emergence of males (Monceau et al. 2014; Darrouzet et al.  
110 2015; Rome et al. 2015), while workers are still present inside the nest. Thus, males are likely to  
111 encounter and interact with female nestmates of both castes inside their colonies. Since mating with  
112 a related nestmate female may be detrimental for males and the two female caste greatly differ in  
113 their reproductive potential, with only reproductive gynes providing males with a return in terms of  
114 fitness, we might expect *V. velutina nigrithorax* males to prefer non-nestmate females over  
115 nestmates and to distinguish between female castes preferring bigger reproductive gynes compared  
116 to sterile workers (Liebert et al. 2010; Cappa et al. 2013; Beani et al. 2014).

117 Given the recent arrival and rapid spread of the species starting from a very low number of  
118 individuals (Arca 2012), disentangling the variables influencing the mating behaviour of the  
119 yellow-legged hornet might provide useful insights into the mechanisms regulating reproduction in  
120 an alien species in the first phases of the invasion process, which, hopefully, might help in  
121 preventing or reducing its spread.

122

## 123 MATERIALS AND METHODS

### 124 *Sample collection*

125 *V. velutina nigrithorax* males and females issued from laboratory reared combs belonging to  
126 four different field colonies located at least 9 Km apart. Combs were collected in Liguria region in  
127 Autumn 2015 and 2016. Combs were transferred to the laboratory and maintained under standard  
128 laboratory condition (natural daylight cycle, 25 °C) in closed cages. Everyday newly emerged  
129 adults were sexed and transferred into separate male and females exclusive glass cages (size 15 ×  
130 15 × 15 cm) where they were fed with ad libitum water and sugar until behavioural tests. Each cage

131 contained a comb fragment (around 20 empty cells) from the hornets' nest of origin to allow the  
132 newly emerged individuals to acquire the chemical cues and form a template for nestmate  
133 recognition as occurs in other social wasps (Gamboa et al. 1986; Signorotti et al. 2014). Daily  
134 removal of newly emerged hornets guaranteed male and female virginity as well as knowledge of  
135 adult age.

136

### 137 *Behavioural assays*

138 We assessed male attraction toward females by comparing interest of groups of focal males  
139 towards different stimuli: (a) nestmate female, (b) non-nestmate female, (c) non-nestmate male as  
140 control. Focal male groups were transferred to a transparent plastic experimental cage (size 20 × 13  
141 × 12 cm). Cages had a round Ø 6 cm steel mesh (1.5 × 1.5 mm) at the centre of the top roof, 3 cm  
142 from the cage sides. Groups consisted of five nestmate males, aged between 10 and 20 days, in  
143 order to have sexually mature males (see Poidatz et al. 2017). After 10 min of habituation, the  
144 stimulus, i.e. female or male hornet, was introduced in a Ø 2.5 × 5 cm plastic tube over the mesh  
145 and the trial started. Stimuli were free to move within the tube during the trial and mesh density (1.5  
146 × 1.5 mm) allowed male antennae to stick through and directly come into contact with the stimulus  
147 body on the other side of the screen. Focal males were presented with one of three stimuli (a, b or  
148 c) and their antennation rate under the stimulus was evaluated. We chose antennation as a clear sign  
149 of male interest towards the presented stimulus (Cappa et al. 2013). All the individuals used as  
150 stimuli (N = 56: 15 males, 41 females) were apparently healthy when tested. Males were used from  
151 1 to 3 times; females used from 1 to 5 times.

152 As an index of male interest towards the different stimuli, we recorded the number of males  
153 antennating in the Ø 2.5 cm circle of wire mesh under the stimulus every minute for 10 min. An  
154 (antennation score, AS: total number of males observed antennating during the 10-min trial as the  
155 sum of antennating males at each of the 10 checks per trial; AS score could range from 0, no male  
156 antennating during checks, to 50, all five males antennating at each check). After 10 min the

157 stimulus was removed. Trials were performed during the central hours of the day (11 am—3 pm)  
158 when males were more active, in October, when both workers and reproductive gynes are present in  
159 the nests (Monceau et al. 2014; Rome et al. 2015). Overall we tested 29 five-male groups,  
160 belonging to 3 colonies, for a total of 172 trials of 10 min. Each group was tested in a random order  
161 with all the stimuli.

162

### 163 *Female caste assessment*

164 After behavioural trials, females used as stimuli (N = 41) were weighted on KERN PCB  
165 350-3 scale, dissected under a Wild M5A stereomicroscope and the presence of fat bodies was  
166 assessed. In *V. velutina* female caste is hard to recognize and morphological differences between  
167 castes have been expressed in various ways (Rome et al. 2015; Pérez-de-Heredia et al. 2017). Some  
168 authors have reported differences linked to the capability of gynes to overwinter using female  
169 weight, both wet and dry, and fat storage to distinguish between workers and gynes (Monceau et al.  
170 2014; Rome et al. 2015). Also the mesoscutum width (MW), from tegula to tegula. is often used as  
171 parameter to discriminate castes in some Vespidae species (Noll et al. 2004; Felippotti et al. 2009;  
172 Felippotti et al. 2010; Pérez-de-Heredia et al. 2017). Given the relative degree of uncertainty linked  
173 to each single measure, we decided to combine different parameters (see below) by using weight  
174 and fat storage alongside MW (index of overall linear size) of each female stimulus as potential  
175 indicators of caste (Noll & Zucchi 2002; Ohl & Thiele 2007; Beani et al. 2011; Pérez-de-Heredia et  
176 al. 2017).

177

### 178 *Statistical analysis*

179 In order to account for the non-independence of data we used a generalized estimating  
180 equations (GEE) approach, which extends the generalized linear model in order to allow the  
181 analysis of correlated observations, such as occurs for clustered data (Burton et al 1998). Moreover

182 GEE are robust even when the error structure model is unknown and/or misspecified, and are more  
183 relaxed on distributional assumptions (Overall & Tonidandel 2004; Hubbard et al. 2010). For both  
184 GEEs, we selected the final model on the basis of the “quasi-likelihood under the independence  
185 model” criterion QIC, by choosing the model parameters that provided the smallest QIC (Pan  
186 2001).

187 We first assessed whether male were more attracted by females (i.e. higher number of  
188 antennations below female stimulus) than by male stimuli. We did it by using a GEE with the  
189 following parameters: antennation score as dependent variable; Negative binomial distribution with  
190 log link function; independent working correlation matrix; fixed effect: stimulus sex; subject effect:  
191 focal male group, stimulus identity, and nest trial (repeated trials for the different focal male  
192 groups) as intra-subject effect.

193 We then focused only on trials with female stimuli to assess the importance of individual  
194 weight, mesoscutum width, presence/absence of fat bodies and nestmateship (i.e. whether the  
195 stimulus came from the same colony of the focal males). As individual weight, mesoscutum width,  
196 presence/absence of fat bodies were positively correlated (Pearson correlation test, Weight X  
197 Mesoscutum width:  $r = 0.862$ ,  $P < 0.001$ ,  $n = 37$ ; Weight X Fat body:  $r = 0.786$ ,  $P < 0.001$ ,  $n = 37$ ;  
198 Mesoscutum width X Fat body:  $r = 0.724$ ,  $P < 0.001$ ,  $n = 37$ ; Fig. 1) we used principal component  
199 analysis to create a single predictor explaining most variance of the three original individual  
200 features (86.1% of variance explained, Kaiser-Meyer-Olkin Measure of Sampling Adequacy =  
201 0.727) retaining the first principal components (hereafter female physical condition, FPC). Weight,  
202 mesoscutum width, presence/absence of fat bodies had loadings of 0.953, 0.930, 0.899  
203 respectively). We used the following model parameters in GEE: antennation score as dependent  
204 variable; Negative binomial distribution with log link function; independent working correlation  
205 matrix; fixed effect: female physical condition, nestmateship and the interaction between  
206 nestmateship and female physical condition; subject effect: focal male group, stimulus identity and



207 nest trial as intra-subject effect. All analyses used a model-based estimator and a type III analysis.  
208 Statistical Analyses were performed in SPSS 20.0 (SPSS 2011).

209

210

## RESULTS

211 Overall antennation response was rather low, with at least one antennation present only in  
212 the 34 % of the tests. Antennation score was higher when the stimulus was female than when it was  
213 a male (Wald  $\chi^2 = 21.111$ ,  $df = 1$ ,  $P < 0.001$ , estimated marginal means  $\pm$  SE: Female stimuli 2.212  
214  $\pm 0.220$ ; male stimuli:  $0.292 \pm 0.125$ ). When considering only trials with female stimuli,  
215 antennation score was significantly affected by female physical condition (Wald  $\chi^2 = 69.329$ ,  $df = 1$ ,  
216  $P = 0.002$ ) with heavier, larger females with fat bodies evoking a higher antennation response (Fig.  
217 2). Neither nestmateship nor the interaction between nestmateship and female physical condition  
218 were significant (respectively Wald  $\chi^2 = 1.688$ ,  $df = 1$ ,  $P = 0.194$ ; Wald  $\chi^2 = 2.206$ ,  $df = 1$ ,  $P =$   
219  $0.137$ ).

220

221

## DISCUSSION

222 Our results demonstrated that males respond differentially to the presented stimuli showing  
223 a higher interest towards those females which could provide the highest potential return in terms of  
224 fitness, regardless of nestmateship.

225 As expected, males in our laboratory bioassays were more attracted to females than males,  
226 preferring bigger heavier females, with more abundant fat storage, compared to smaller ones.  
227 Vespinae wasps usually show morphological differences between female castes, with reproductive  
228 females being larger than workers (Jeanne & Suryanarayanan 2011). However, not all species  
229 present the same degree of caste differentiation. In the genus *Vespa* some species, such as *Vespa*  
230 *mandarinia*, *V. affinis*, *V. crabro* or *V. simillima*, have castes characterized by clear size separation.  
231 By contrast, hornets like *V. tropica* and *V. analis*, show an overlap of caste sizes (Matsuura &  
232 Yamane 1990). Also in *V. velutina nigrithorax*, female caste is hard to recognize (Pérez-de-Heredia

233 et al. 2017), however, as in other temperate social wasps (Spradberry 1973; Strassmann et al 1984;  
234 O'Donnell 1998; Toth et al. 2009; Perrard et al. 2012) reproductive gynes, that are the only females  
235 surviving winter, are usually bigger and show more abundant fat bodies, whereas smaller workers,  
236 that do not overwinter, have very scarce or absent fat bodies. Female body size, weight and fat  
237 storage appear therefore as reliable indicators of female caste (Strassmann et al 1984; Hunt et al.  
238 2007, 2010; Cervo et al. 2008; Toth et al. 2009). Thus, the observed male preference for bigger  
239 heavier females, with more abundant fat storages, might due to the fact that such females are the  
240 more likely to survive the winter diapause and found a new colony the following season, as in other  
241 Vespidae species (Hunt et al. 2007, 2010; Cervo et al. 2008), and we may expect males to have  
242 evolved accurate systems to detect the most suitable partners. Moreover, it seems likely that  
243 reproductive females could actively signal their condition, while there might be a lack of male-  
244 attractive signal production by non-reproductive workers (Wen et al. 2017); thus, variability in  
245 female signal production may underlies the difference in male interest response towards the two  
246 female castes.

247         Due to the conditions of our laboratory set-up, we were able to highlight mainly short-range  
248 attractiveness. The observed male attraction towards putative reproductive females could be due to  
249 short-range chemical cues, such as cuticular hydrocarbons (CHCs), which can be perceived at  
250 distances of few cm (Ferveur 2005; Saïd et al. 2005). It is possible that males walking around the  
251 cage could sense the cuticular blend of receptive females inside the tubes through the separating  
252 mesh. Indeed, CHCs of *V. velutina nigrithorax* differ according to caste and sex (Gévar et al. 2017).  
253 Also in the European hornet, *Vespa crabro*, males are attracted by caged reproductive gynes or  
254 workers treated with gynes extracts, suggesting the cuticular alkenes and methyl-branched  
255 compounds, different between the two castes, as short-range sexual attractants (Spiewok et al.  
256 2006).

257         Contrary to expectations, our males did not show any preference for non-nestmate females  
258 over nestmates ones. This lack of preference together with the alleged intra-nest mating could

259 explain the very low genetic diversity and high level of male diploidy observed inside *V. velutina*  
260 *nigrithorax* colonies in the European invasive range (Monceau et al. 2014; Darrouzet et al. 2015). If  
261 mating takes place, at least in part, inside the nest, males may rely on CHCs as chemical cues to  
262 identify receptive females. Such hypothesis is corroborated by the fact that yellow-legged hornet  
263 males present an olfactory subsystem for CHCs perception similar to females (Couto et al. 2017).  
264 However, also long-range attractants, such as the putative sex pheromone recently identified in the  
265 other *V. velutina* Asian subspecies (Wen et al. 2017), could be at work in the attraction process. In  
266 fact, males might leave the nest in order to find receptive gynes attracting them away from their  
267 nests via sex pheromones (Wen et al. 2017). Thus, males would not need to discriminate female  
268 caste and nestmateship in order to find the most suitable partner. Although we did not observed any  
269 clear sign of immediate attraction towards the preferred females, which could be expected in the  
270 case of rapidly spreading volatile sex attractants, it is possible that our laboratory setting may have  
271 influenced the results. Indeed, both female and male hornets in our behavioural assays emerged in  
272 the lab and they did not experience field conditions. This might have had an impact on their sexual  
273 behaviour, which might require other triggers that we were not able to reproduce under laboratory  
274 conditions, especially if males leave the nest in search for receptive females emitting sex  
275 pheromones (Wen et al. 2017). Finally, inbreeding avoidance could also be the result of a separate  
276 decision that occurs after attraction. Males might be attracted via a long-distance sex pheromone,  
277 but then copulation could be inhibited by nestmate chemical or behavioural cues upon the initiation  
278 of mating behaviour, therefore avoiding inbreeding. Although males were able to contact the body  
279 of the stimuli through the mesh, they could not directly interact with them, it is therefore possible  
280 that behavioural interactions between the two potential partners might prevent or favour successful  
281 copulations (e.g. females might actively reject mating attempts from nestmate males).

282 Overall, our findings deepen the knowledge on the reproductive behaviour of this invasive  
283 species investigating for the first time the extent of male sexual preference by *V. velutina*  
284 *nigrithorax* males. Our results show that males from an invasive population of *V. velutina*

285 *nigrithorax* do not seem capable of nestmate recognition in a sexual context. We might hypothesize  
286 that in the invasive population the chemical distance among nests' signature mixtures, although  
287 present (Gévar et al. 2017) may be less marked than in the native population because of the high  
288 rate of inbreeding, not providing males with the cues needed for nestmate discrimination. Such  
289 hypothesis, however, is unlikely, since the chemical signature of the invasive population of *V.*  
290 *velutina nigrithorax* is quite heterogeneous with each colony, caste, and gender characterized by a  
291 specific CHC profile (Gévar et al. 2017). Alternatively males could have not evolved the ability to  
292 recognize nestmates when choosing a mate in their invasive range: in native populations, nestmate  
293 recognition at natural mating sites may not be of great importance due to a lower probability of  
294 encountering relatives in the field where females seem to attract males from a distance via sex  
295 pheromones (Wen et al. 2017). However, the absence of inbreeding avoidance by males and the  
296 consequent costs could be counterbalanced by the relative degree of polyandry recorded in *V.*  
297 *velutina nigrothorax* queens, which could compensate the low genetic diversity due to the single  
298 introduction event and inbreeding inside nests (Arca 2012; Monceau et al. 2014). Comparative  
299 studies examining acceptance thresholds for mating with relatives and chemical signature  
300 differences among colonies across native and invasive populations would help illuminate whether  
301 colonizing populations do indeed shift their mate acceptance thresholds in a new environment.  
302 Overall, our findings, although limited by the laboratory settings, give new insights in the  
303 understanding of the largely unknown reproductive biology of the yellow-legged hornet in its  
304 invasive range. Further research should aim at investigating male attraction also in the field, even if  
305 the task may not be easy since current management practice call for an early detection and  
306 destruction of nests.

307

308

#### ACKNOWLEDGEMENTS

309 The authors greatly thank Fabrizio Zagni and Nuccio Lanteri for their help in collecting hornet  
310 colonies in the field.

311

312

## FUNDING

313 Financial support was provided by the project ALIEM (Action pour Limiter les risques de diffusion  
314 des espèces Introduites Envahissantes en Méditerranée), funded by the ‘Programma Interreg Italia-  
315 Francia Marittimo 2014-2020. La cooperazione al cuore del Mediterraneo’, axe II. A. Cini was  
316 funded by a Marie Sklodowska-Curie Action, grant 539176.

317

318

## DISCLOSURE STATEMENT

319 No potential conflict of interest was reported by the authors.

320

321

## ETHICAL STANDARD

322 All procedures involving any experimental animals were performed in compliance with local  
323 animal welfare laws.

324

325

## AUTHOR CONTRIBUTION

326 F. Cappa, A. Cini, I. Pepiciello and R. Cervo conceived and designed research. A. Cini, F. Cappa, I.  
327 Pepiciello and I. Petrocelli performed the assays and collected the data. A. Cini analyzed the data.  
328 F. Cappa and A. Cini wrote the manuscript. All authors read and approved the manuscript. F.  
329 Cappa, A. Cini and I. Pepiciello contributed equally to this work.

330

331

332

333

## REFERENCES

334 Allendorf FW, Lundquist LL. 2003. Introduction: population biology, evolution, and control of  
335 invasive species. *Conserv Biol.* 17:24-30.

336

337 Arca M. 2012. Caractérisation génétique et étude comportementale d'une espèce envahissante en  
338 France: *Vespa velutina* Lepeletier (Hymenoptera, Vespidae). [Genetic and behavioural  
339 characterization of an invasive species in France: *Vespa velutina* Lepeletier (Hymenoptera,  
340 Vespidae)] [PhD dissertation]. Paris: Université Pierre et Marie Curie. French.

341

342 Beani L, Dallai R, Mercati D, Cappa F, Giusti F, Manfredini F. 2011. When a parasite breaks all the  
343 rules of a colony: morphology and fate of wasps infected by a strepsipteran endoparasite. *Anim*  
344 *Behav.* 82:1305-1312.

345

346 Beani L, Dessì-Fulgheri F, Cappa F, Toth A. 2014. The trap of sex in social insects: from the  
347 female to the male perspective. *Neurosci Biobehav Rev.* 46:519-533.

348

349 Beggs JR, Brockerhoff EG, Corley JC, Kenis M, Masciocchi M, Muller F, Rome Q, Villemant C.  
350 2011. Ecological effects and management of invasive alien Vespidae. *BioControl.* 56:505-526.

351

352 Burton P, Gurrin L, Sly P. 1998. Tutorial in biostatistics. Extending the simple linear regression  
353 model to account for correlated responses: an introduction to generalized estimating equations and  
354 multi-level mixed modeling. *Stat Med.* 17:1261-1291.

355

356 Cappa F, Bruschini C, Cervo R, Turillazzi S, Beani L. 2013. Males do not like the working class:  
357 male sexual preference and recognition of functional castes in a primitively eusocial wasp. *Anim*  
358 *Behav.* 86:801-810.

359

360 Cervo R, Dapporto L, Beani L, Strassmann JE, Turillazzi S. 2008. On status badges and quality  
361 signals in the paper wasp *Polistes dominulus*: body size, facial colour patterns and hierarchical rank.  
362 Proc R Soc B. 275:1189-1196.

363

364 Chapin FS III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU,  
365 Lavorel S, Sala OE, Hobbie SE, et al. 2000. Consequences of changing biodiversity. Nature.  
366 405:234-242.

367

368 Chapman RE, Bourke AFG. 2001. The influence of sociality on the conservation biology of social  
369 insects. Ecol Lett. 4:650-662.

370

371 Choi MB, Martin SJ, Lee JW. 2012. Distribution, spread, and impact of the invasive hornet *Vespa*  
372 *velutina* in South Korea. J Asia Pac Entomol. 15:473-477.

373

374 Cini A, Cappa F, Petrocelli I, Pepiciello I, Bortolotti L, Cervo R. 2018. Competition between the  
375 native and the introduced hornets *Vespa crabro* and *Vespa velutina*: a comparison of potentially  
376 relevant life-history traits. Ecol Entomol. doi:<https://doi.org/10.1111/een.12507>

377

378 Couto A, Mitra A, Thiéry D, Marion-Poll F, Sandoz JC. 2017. Hornets have it: a conserved  
379 olfactory subsystem for social recognition in Hymenoptera? Front Neuroanat. 11:48.

380

381 Crozier RH. 1971. Heterozygosity and sex determination in haplodiploidy. Am Nat. 105:399-412.

382

383 Darrouzet E, Gévar J, Guignard Q, Aron S. 2015. Production of early diploid males by European  
384 colonies of the invasive hornet *Vespa velutina nigrithorax*. PloS ONE. 10:e0136680.  
385

386 Davidson AM, Jennions M, Nicotra AB. 2011. Do invasive species show higher phenotypic  
387 plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol Lett. 14:419-431.  
388

389 Felippotti GT, Mateus L, Mateus S, Noll FB, Zucchi R. 2010. Morphological caste differences in  
390 three species of the neotropical genus *Clypearia* (Hymenoptera: Polistinae: Epiponini). Psyche.  
391 doi:10.1155/2010/410280  
392

393 Felippotti GT, Tanaka Junior GM, Noll FB, Wenzel JW. 2009. Discrete dimorphism among castes  
394 of the bald-faced hornet *Dolichovespula maculata* (Hymenoptera: Vespidae) in different phases of  
395 the colony cycle. J Nat Hist. 43:2481-2490  
396

397 Ferveur JF. 2005 Cuticular hydrocarbons: Their evolution and roles in *Drosophila* pheromonal  
398 communication. Behav Genet. 35:279-295.  
399

400 Fonseca DM, LaPointe DA, Fleischer RC. 2000. Bottlenecks and multiple introductions: population  
401 genetics of the vector of avian malaria in Hawaii. Mol Ecol. 9:1803-1814.  
402

403 Frankham R. 2005. Resolving the genetic paradox in invasive species. Heredity. 94:385-385.  
404

405 Gallai N, Salles J-M, Settele J, Vaissiere BE. 2009. Economic valuation of the vulnerability of  
406 world agriculture confronted with pollinator decline. Ecol Econ. 68:810-821.



407 Gamboa GJ, Reeve HK, Pfennig DW. 1986. The evolution and ontogeny of nestmate recognition in  
408 social wasps. *Annu Rev Entomol.* 31:431-454.

409

410 Gévar J, Bagnères AG, Christidès JP, Darrouzet E. 2017. Chemical heterogeneity in inbred  
411 European population of the invasive hornet *Vespa velutina nigrithorax*. *J Chem Ecol.* 43:763-777.

412

413 Hingston AB, McQuillan PB. 1999. Displacement of Tasmanian native megachilid bees by the  
414 recently introduced bumblebee *Bombus terrestris* (Linnaeus, 1758) (Hymenoptera: Apidae). *Aust J*  
415 *Zool.* 47:59-65.

416

417 Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ. 2002. The causes and consequences of ant  
418 invasions. *Annu Rev Ecol Sys.* 33:181-233.

419

420 Hubbard AE, Ahern J, Fleischer NL, Van der Laan, M, Lippman SA, Jewell N, Bruckner T,  
421 Satariano WA. 2010. To GEE or not to GEE: comparing population average and mixed models for  
422 estimating the associations between neighborhood risk factors and health. *Epidemiology.* 21:467-  
423 474.

424

425 Hunt JH, Kensinger BJ, Kossuth JA, Henshaw MT, Norberg K, Wolschin F, Amdam GV. 2007. A  
426 diapause pathway underlies the gyne phenotype in *Polistes* wasps, revealing an evolutionary route  
427 to caste-containing insect societies. *Proc Natl Acad Sci USA.* 104:14020-14025.

428

429 Hunt JH, Wolschin F, Henshaw MT, Newman TC, Toth AL, Amdam GV. 2010. Differential gene  
430 expression and protein abundance evince ontogenetic bias toward castes in a primitively eusocial  
431 wasp. *PLoS ONE.* 5:e10674.

432

433 Inoue MN, Yokoyama J, Washitani I. 2008. Displacement of Japanese native bumblebees by the  
434 recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *J Insect Conserv.* 12:135-146.

435

436 Jeanne RL, Suryanarayanan S. 2011. A new model for caste development in social wasps. *Commun*  
437 *Integr Biol.* 4:373-377.

438

439 Lee CE. 2002. Evolutionary genetics of invasive species. *Trend Ecol Evol.* 17:386-391.

440

441 Levine JM, D'antonio CM. 2003. Forecasting biological invasions with increasing international  
442 trade. *Conserv. Biol.* 17:322-326.

443

444 Liebert AE, Johnson RN, Switz GT, Starks PT. 2004. Triploid females and diploid males:  
445 Underreported phenomena in *Polistes* wasps? *Insectes Soc.* 51:205–211.

446

447 Liebert AE, Wilson-Rich N, Johnson CE, Starks PT. 2010. Sexual interactions and nestmate  
448 recognition in invasive populations of *Polistes dominulus* wasps. *Insectes Soc.* 57:457-463.

449

450 Liu Z, Chen S, Zhou Y, Xie C, Zhu B, Zhu H, Liu S, Wang W, Chen H, Ji Y. 2015. Deciphering  
451 the venom transcriptome of killer-wasp *Vespa velutina*. *Sci Rep.* 5:9454.

452

453 Lockwood JL, Cassey P, Blackburn T. 2005. The role of propagule pressure in explaining species  
454 invasions. *Trend Ecol Evol.* 20:223-228.

455

456 Lonsdale WM. 1999. Global patterns of plant invasions and the concept of invasibility. *Ecology*.  
457 80:1522-1536.

458

459 Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. 100 of the world's worst invasive alien  
460 species: a selection from the global invasive species database. Auckland: Invasive Species  
461 Specialist Group.

462

463 Matsuura M, Yamane S. 1990. *Biology of the vespine wasps*. Springer Verlag.

464

465 Mehta SV, Haight RG, Homans FR, Polasky S, Venette RC. 2007. Optimal detection and control  
466 strategies for invasive species management. *Ecol Econom*. 61:237-245.

467

468 Moller H. 1996. Lessons for invasion theory from social insects. *Biol Conserv*. 78:125-142.

469

470 Monceau K, Bonnard O, Thiéry D. 2014. *Vespa velutina*: a new invasive predator of honeybees in  
471 Europe. *J Pest Sci*. 87:1-16.

472

473 Mooney HA, Cleland EE. 2001. The evolutionary impact of invasive species. *Proc Natl Acad Sci*.  
474 98:5446-5451.

475

476 Noll FB, Zucchi, R. 2002. Castes and the influence of the colony cycle in swarm-founding polistine  
477 wasps (Hymenoptera, Vespidae, Epiponini). *Insectes Soc*. 49:62-74.

478

479 Noll FB, Wenzel JW, Zucchi R. 2004. Evolution of caste in neotropical swarm-founding wasps  
480 (Hymenoptera: Vespidae; Epiponini). *Am Mus Novit.* 3467:1-24.  
481

482 O'Donnell S. 1998. Reproductive caste determination in eusocial wasps (Hymenoptera: Vespidae).  
483 *Annu Rev Entomol.* 43:323-346.  
484

485 Ohl M, Thiele K. 2007. Estimating body size in apoid wasps: the significance of linear variables in  
486 a morphologically diverse taxon (Hymenoptera, Apoidea). *Mitt Zool Mus Berl.* 2:110–124.  
487

488 Overall JE, Tonidandel S. 2004. Robustness of generalized estimating equation (GEE) tests of  
489 significance against misspecification of the error structure model. *Biometr J.* 46:203-213.  
490

491 Paini DR, Dale Roberts J. 2005. Commercial honey bees (*Apis mellifera*) reduce the fecundity of an  
492 Australian native bee (*Hylaeus alcyoneus*). *Biol. Conserv.* 123:103-112.  
493

494 Pan W. 2001. Akaike's information criterion in generalized estimating equations. *Biometrics.*  
495 57:120-125.  
496

497 Pejchar L, Mooney HA. 2009. Invasive species, ecosystem services and human well-being. *Trend*  
498 *Ecol Evol.* 24:497-504.  
499

500 Pérez-de-Heredia I, Darrouzet E, Goldarazena A, Romón P, Iturrondobeitia JC. 2017.  
501 Differentiating between gynes and workers in the invasive hornet *Vespa velutina* (Hymenoptera,  
502 Vespidae) in Europe. *J Hymenopt Res.* 60:119-133.

503

504 Perrard A, Villemant C, Carpenter JM, Baylac M. 2012. Differences in caste dimorphism among  
505 three hornet species (Hymenoptera: Vespidae): forewing size, shape and allometry. *J Evol Biol.*  
506 25:1389-1398.

507

508 Poidatz J, Bressac C, Bonnard O, Thiéry D. 2017. Delayed sexual maturity in males of *Vespa*  
509 *velutina*. *Insect Sci.* doi:10.1111/1744-7917.12452

510

511 Pyšek P, Richardson DM. 2010. Invasive species, environmental change and management, and  
512 health. *Annu Rev Env Resour.* 35:25-55.

513

514 Rasner CA, Yeh P, Eggert LS, Hunt KE, Woodruff DS, Price TD. 2004. Genetic and morphological  
515 evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*. *Mol Ecol.*  
516 13:671-681

517

518 Rome Q, Muller FJ, Touret-Alby A, Darrouzet E, Perrard A, Villemant C. 2015. Caste  
519 differentiation and seasonal changes in *Vespa velutina* (Hym.: Vespidae) colonies in its introduced  
520 range. *J Appl Entomol.* 139:771-782.

521

522 Rortais, A, Villemant C, Gargominy O, Rome Q, Haxaire J, Papachristoforou A, Arnold G. 2010. A  
523 new enemy of honeybees in Europe: The Asian hornet *Vespa velutina*. In: Settele J, et al., editors.  
524 Atlas of biodiversity risks. From Europe to globe, from stories to maps. Sofia & Moscow: Pensoft  
525 Publisher; p.181.

526

527 Ross KG, Carpenter JM. 1991. Population genetic structure, relatedness, and breeding systems. In:  
528 Ross KG, Matthews RW, editors. The social biology of wasps. Ithaca and London: Comstock  
529 Publishing Associates; p. 451-479.

530

531 Saïd I, Gaertner C, Renou M, Rivault C. 2005. Perception of cuticular hydrocarbons by the  
532 olfactory organs in *Periplaneta americana* (L.) (Insecta: Dictyoptera). J Insect Physiol. 51:1384-  
533 1389.

534

535 Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ,  
536 Cohen JE, Ellstrand NC, et al. 2001. The population biology of invasive species. Annu Rev Ecol  
537 System. 32:305-332.

538

539 Shah FA, Shah TA. 1991. *Vespa velutina*, a serious pest of honeybees in Kashmir. Bee World.  
540 72:161-164.

541

542 Signorotti L, Cappa F, d’Ettorre P, Cervo R. 2014. Novel insights into the ontogeny of nestmate  
543 recognition in *Polistes* social wasps. PloS ONE. 9:e97024.

544

545 Spiewok S, Schmolz E, Ruther J. 2006. Mating system of the European hornet *Vespa crabro*: male  
546 seeking strategies and evidence for the involvement of a sex pheromone. J Chem Ecol. 32:2777-  
547 2788.

548

549 Spradberry JP. 1973. Wasps: An account of the biology and natural history of solitary and social  
550 wasps with particular reference to those of the British Isles. London (UK): Sidgwick and Jackson.

551

552 SPSS I. 2011. IBM SPSS statistics for Windows, version 20.0. New York: IBM Corp.

553

554 Starks PT, Fischer DJ, Watson RE, Melikian GL, Nath SD. 1998a. Context-dependent nestmate  
555 discrimination in the paper wasp, *Polistes dominulus*: A critical test of the optimal acceptance  
556 threshold model. Anim Behav. 56:449-458.

557

558 Starks PT, Watson RE, Dipaola MJ, Dipaola CP. 1998b. The effect of queen number on nestmate  
559 discrimination in the facultatively polygynous ant *Pseudomyrmex pallidus* (Hymenoptera:  
560 Formicidae). Ethology. 104:573-584.

561

562 Strassmann JE, Lee RE, Rojas RR, Baust JG. 1984. Caste and sex differences in cold-hardiness in  
563 the social wasps, *Polistes annularis* and *P. exclamans* (Hymenoptera: Vespidae). Insectes Soc.  
564 31:291-301.

565

566 Tan K, Radloff S, Li J, Hepburn H, Yang M-X, Zhang L, Neumann P. 2007. Bee-hawking by the  
567 wasp, *Vespa velutina*, on the honeybees *Apis cerana* and *A. mellifera*. Naturwissenschaften. 94:469-  
568 472.

569

570 Toth AL, Bilof KBJ, Henshaw MT, Hunt JH, Robinson GE. 2009. Lipid stores, ovary development,  
571 and brain gene expression in *Polistes metricus* females. Insect Soc. 56:77-84.

572

573 Tsutsui ND, Suarez AV, Holway DA, Case TJ. 2000. Reduced genetic variation and the success of  
574 an invasive species. Proc Natl Acad Sci USA. 97:5948-5953.

575

576

577 van Wilgenburg E, Driessen G, Beukeboom LW. 2006. Single locus complementary sex  
578 determination in Hymenoptera: an “unintelligent” design? *Front Zool.* 3:1-15.

579

580 Villemant C, Muller F, Hauboiss S, Perrard A, Darrouzet E, Rome Q. 2011. Bilan des travaux  
581 (MNHN et IRBI) sur l’invasion en France de *Vespa velutina*, le frelon asiatique prédateur  
582 d’abeilles. In: Barbançon J-M, L’Hostis M, editors. *Journée Scientifique Apicole*. Nantes (France):  
583 Oniris-Fnosad, Arles; p. 3–12.

584

585 Wen P, Cheng YN, Dong SH, Wang ZW, Tan K, Nieh JC. 2017. The sex pheromone of a globally  
586 invasive honey bee predator, the Asian eusocial hornet, *Vespa velutina*. *Sci Rep.* 7:12956.

587

588 Whiting PW. 1933. Selective fertilization and sex-determination in Hymenoptera. *Science.* 78:537–  
589 538.

590

591 Whiting PW. 1943. Multiple alleles in complementary sex determination of *Habrobracon*.  
592 *Genetics.* 28:365–382.

593

594

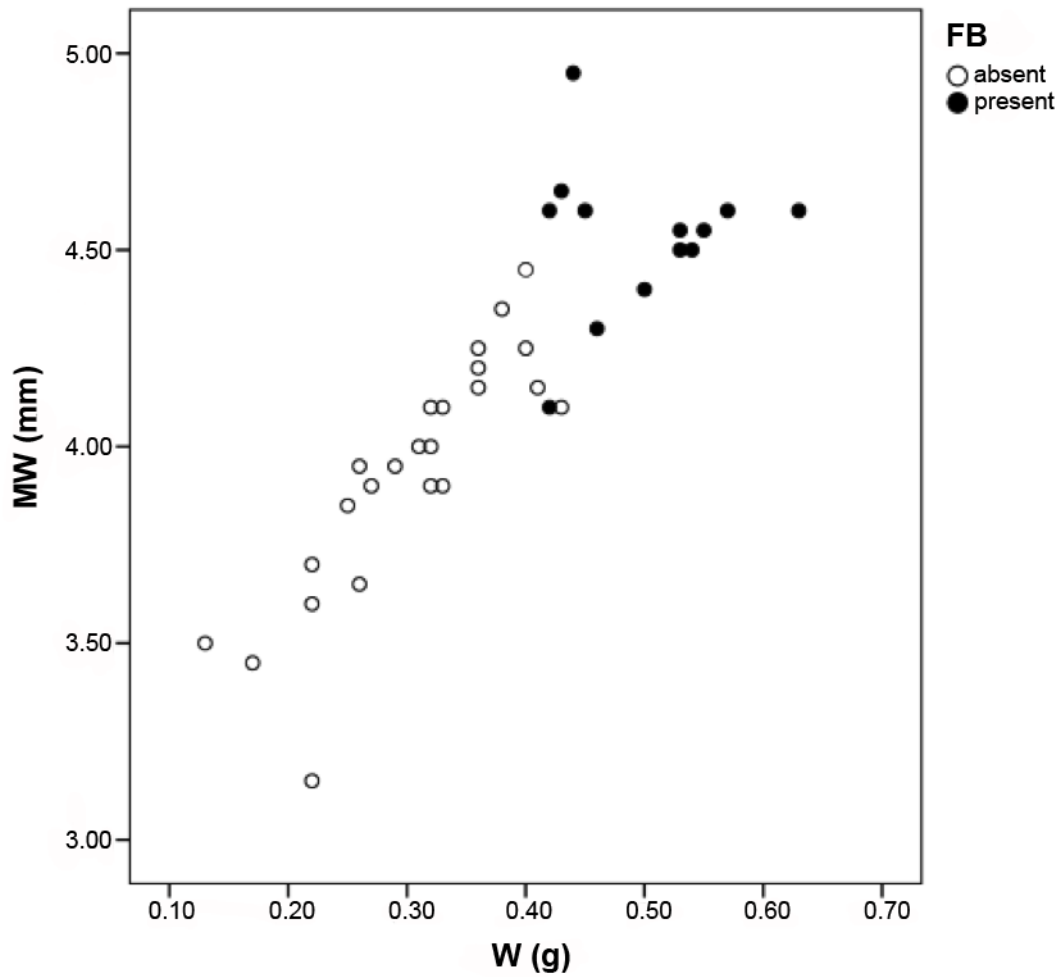
595 Fig. 1. — Correlation between weight (W), presence versus absence of fat bodies (FB) and  
596 mesoscutum width (MW) in female hornets used as stimuli.

597



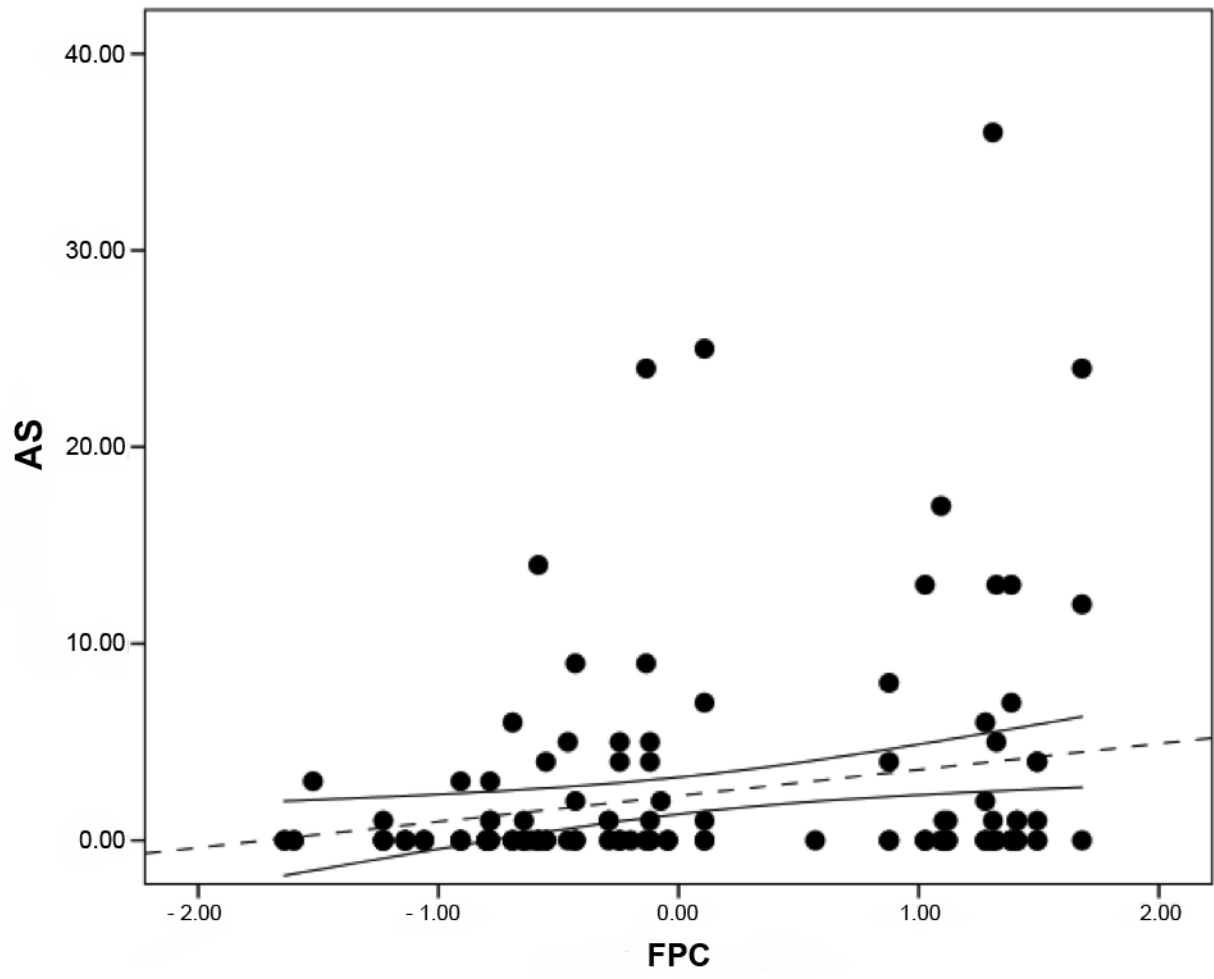
598 Fig. 2. — Differences in antennation score (AS) by focal males according to the physical condition  
599 (defined as in the text, higher values of FPC means heavier, larger females with more developed fat  
600 bodies) of the females presented as stimuli. Solid and dotted lines respectively represent linear  
601 regression line and 95% confidence interval.

602



603

604 Fig.1



605

606 Fig.2