Brood size and sex ratio in response to host quality and wasp traits in the gregarious parasitoid *Oomyzus sokolowskii* (Hymenoptera: Eulophidae)

Xianwei Li [1] , **Liangting Zhu** [1] , **Ling Meng** [1] , **Baoping Li** Corresp. [1]

[1] School of Plant Protection, Nanjing Agricultural University, No. 1 Weigang Rd, Nanjing, Jiangsu 210095, China

Corresponding Author: Baoping Li
Email address: lbp@jnau.edu.cn

This laboratory study investigated whether the larval-pupal parasitoid *O omyzus sokolowskii* females adjust their brood size and sex ratio in response to body size and stage of *Plutella xylostella* larval hosts, as well as to their own body size and the order of oviposition. These factors were analyzed using multiple regression with simultaneous entry of them and their two-way interactions. Parasioid brood size tended to increase with host body size at parasitism when the 4th instar larval host was attacked, but did not change when the 2nd and 3rd instar larvae were attacked. Parasitoids did not vary in brood size according to their body size, but decreased with their bouts of oviposition on a linear trend from 10 offspring adults emerged per host in the first bout of oviposition down to 8 in the third. Parasitoid offspring sex ratio did not change with host instar, host body weight, wasp body size, and oviposition bout. Proportions of male offspring per brood were from 11 % to 13 % from attacking the 2nd to 4th instar larvae and from 13 % to 16 % across three successive bouts of oviposition, with a large variation for smaller host larvae and wasps. When fewer than 12 offspring were emerged from a host, 1 male was most frequently produced; when more than 12 offspring were emerged, 2 and more males were produced. Our study suggests that *O. sokolowskii* females may optimize their clutch size in response to body size of mature *P. xylostella* larvae, and their sex allocation in response to clutch size.

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3 Xianwei Li, Liangting Zhu, Ling Meng, Baoping Li*

4 (School of Plant Protection, Nanjing Agricultural University, No. 1 Weigang Rd, Nanjing, Jiangsu 210095, P.

5 R. China)

10 *** Corresponding author**: Baoping Li

11 School of Plant Protection

12 Nanjing Agricultural University

13 Weigang 1#, Nanjing, Jiangsu Province, Zip code: 210095

14 E-mil: lbp@njau.edu.cn

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19 **ABSTRACT:**

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25 the 2nd and 3rd instar larvae were attacked. Parasitoids did not vary in brood size according to their body size,

26 but decreased with their bouts of oviposition on a linear trend from 10 offspring adults emerged per host in the

27 first bout of oviposition down to 8 in the third. Parasitoid offspring sex ratio did not change with host instar,

28 host body weight, wasp body size, and oviposition bout. Proportions of male offspring per brood were from 11

29 % to 13 % from attacking the 2nd to 4th instar larvae and from 13 % to 16 % across three successive bouts of

30 oviposition, with a large variation for smaller host larvae and wasps. When fewer than 12 offspring were

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32 more males were produced. Our study suggests that *O. sokolowskii* females may optimize their clutch size in

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34

35 **INTRODUCTION**

36 Parasitoids make oviposition decisions depending on a variety of factors, including the quality of hosts and

37   characteristics of ovipositing wasps (Charnov et al., 1981; van Alphen & Visser, 1990; Godfray, 1994; Ellers

38   & Jervis, 2003). Host quality for parasitoid development is often assumed to scale with host size and this

39   pattern is true for idiobiont parasitoids, which arrest host growth at the time of oviposition (Askew & Shaw,

40   1986; King, 1990; Otto & Mackauer, 1998). This pattern, however, may not be universally applicable to

41   koinobiont parasitoids, which allow the host to continue to develop after it has been parasitized (Werren, 1984;

42   Waage, 1986; Mackauer, Sequeira & Otto, 1997; Harvey, Sano & Tanaka, 2010), and thus the future resources

43   for developing koinobiont larvae can vary depending on the host age or development stage at the time of

44   oviposition (Harvey & Strand, 2002; Harvey, 2005), though there are some koinobiont parasitoids still

45   preferring larger body hosts (King, 1989; Elzinga, Harvey & Biere, 2003; Kant, Minor & Trewick, 2012). In

46   addition to host quality, parasitoid oviposition decisions may also depend on characteristics of ovipositing

47   wasps, including their body size and states, such as egg load, age, and nutrition states. The wasp size is

48   frequently considered to be the main target for selection (Godfray, 1994; Ode & Strand, 1995; Bezemer,

49   Harvey & Mills, 2005). It can affect fitness by influencing searching efficiency, longevity, or egg supplies

50   (Mayhew & Heitmans, 2000; Milonas, 2005; Jervis, Ellers & Harvey, 2008; Kasamatsu & Abe, 2015). In

51   addition, it may also influence the quality of host attacked, in that a small female wasp may not be able to use a

52   large, good quality host that is more effective in defense against attacks (Godfray, 1994; Henry, Ma &

53   Roitberg, 2009). Parasitoid state can affect many parasitoid behaviors (van Alphen & Visser, 1990; Roitberg &

54   Bernard, 2008; Harvey, Poelman & Tanaka, 2013). For example, oviposition experience and physiological

55   constraints, such as egg load, can influence parasitoid oviposition decisions (Cloutier et al., 1991; Henneman

56   et al., 1995; Sirot, Ploye & Bernstein, 1997; Ueno, 1999; Rosenheim et al., 2008; Dieckhoff et al., 2014).

57   Therefore, parasitoids may integrate the information provided by a variety of cues about hosts and themselves

58   to make oviposition decisions.

59   *Oomyzus sokolowskii* (Kurdjumov) is a gregarious larval-pupal koinobiont endoparasitoid of the

60   diamondback moth, *Plutella xylostella* (L.) (Talekar & Shelton, 1993; Talekar & Hu, 1996; Silva-Torres et al.,

61   2009), which is one of major herbivorous pests on cruciferous crops (Furlong & Zalucki, 2007; Zalucki et al.,

62   2012; Li et al., 2016). Because *P. xylostella* populations have developed resistance to numerous pesticides (Liu,

63   Tzeng & Sun, 1981; Ferré et al., 1991; Li et al., 2006), biological control with natural enemies offers greater

64   potential (Talekar & Shelton, 1993; Delvare, 2004; Sarfraz, Keddie & Dosdall, 2005). The parasitoid *O.*

65   *sokolowskii* has been found parasitizing *P. xylostella* worldwide (Wang et al., 1999; Nakamura & Noda, 2001;

66   Silva-Torres et al., 2009; Li, Niu & Liu, 2014), showing the potential for biological control of *P. xylostella*

67   (Talekar & Hu, 1996; Furlong, Wright & Dosdall, 2013). For *O. sokolowskii* developmental performances in

68   attacking *P. xylostella* larvae of different instars, two non-choice studies are not consistent in their conclusions,

69   though the female wasp prefers mature larvae under choice circumstances (Talekar & Hu, 1996). While Wang

70   et al. (1999) showed similar performances in the development time, number, and sex ratio of offspring in

71   attacking host larvae of different instars, Nakamura & Noda (2002) showed that *O. sokolowskii* brood size

72   increased with host body size when hosts for parasitism were confined to the prepupal stage. It would be

73   interesting to examine whether or not parasitoid brood size varies as a function of host body size depending on

74   host larval stage at parasitism. In addition, states of ovipositing wasps may influence oviposition decisions. It

75   has been shown that older *O. sokolowskii* females declined in the progeny produced per female and the number

76   of parasitoids emerged per host (Silva-Torres, Barros & Torres, 2009; Sow et al., 2013). However, scant

77   attention has been paid to the effect of *O. sokolowskii* female body size on its oviposition behavior. Therefore,

78   to fully understand *O. sokolowskii* oviposition strategy, we need studies involving both host quality and wasp

79   traits.

80   This laboratory study aimed to answer the question: How do *O. sokolowskii* females make oviposition

81   decisions on brood size and sex ratio in response to both host quality and their own traits? We examined

82   combined effects of body size and age of *P. xylostella* larvae as well as body size and ovipositing order of the

83   female wasp, by using mutiple regression models with simultaneous entry of these factors and their two-way

84   interactions. Our study provides new data to the understanding of oviposition behavior in gregarious

85   parasitoids.

86

87   **MATERIALS & METHODS**

88   **Insects**

89   *Plutella xylostella* was collected in the early spring 2014 from a pakchoi (*Brassica chinensis* L.) field on

90   eastern suburb of Nanjing, Jiangsu, and thereafter maintained on potted pakchoi seedlings in insectary at 25 ±

91   1°C, 60 ± 5 % RH, and a photoperiod of 16 : 8 h (L:D).

92   *Oomyzus sokolowskii* was obtained from the Key Laboratory of Northwest Loess Plateau Crop Pest

93   Management of Ministry of Agriculture of China, Northwest A&F University, Yangling, Shaanxi. It was

94   maintained with *P. xylostella* 4th instar larvae as hosts in the insectary. A group of 4 host larvae was introduced

95   into a 4 ml centrifuge tube, which was covered by 100 mesh plastic screen for ventilation and supplied with a

96   piece of fresh pakchoi leaf as food. Then a female *O. sokolowskii* was released into the tube. After 24 h the

97   larvae were removed from the tube and transferred individually to 10-cm-diameter Petri dishes containing

98   excised fresh pakchoi leaves, which were replaced daily. The host pupae parasitized (different from normal

99   ones by dark body color) were collected in groups in vials, where emerged parasitoid adults were maintained

100   for one day to ensure fully mating. Naive females emerged within one day were used in the experiments.

101   **Oviposition behavior**

102   *Plutella xylostella* 2nd, 3rd and 4th instar larvae were used as hosts. Each larva was individually exposed to

103   parasitism after weighed (Mettler Toledo, AL204-IC, with the measurement accuracy of 0.01 mg). After

104   introduced into a 4 ml centrifuge tube containing a host larva, a female wasp was continuously observed until a

105   bout of oviposition was completed. The wasp then was removed and immediately introduced into another tube

106   containing a same-aged host larva for the second bout of oviposition; then for the third. Three successive bouts

107   of oviposition took ca. 5-6 h to be accomplished by a wasp, which was considered as a replicate. The number

108   of replicates for the 2nd, 3rd, and 4th instar host larvae was 32, 43, and 34, respectively. After the three bouts of

109   oviposition, the wasp was collected and measured in the right hind tibia length (HTL) under the microscope as

110   a measure of body size. The parasitized host larvae were individually reared on excised pakchoi leaves in tubes

111   until parasitoid offspring adults emerged. Emerged wasps from each host were counted as a measure of brood

112   size, and their sexes determined by their antennae with long hairs on each segments in males and without the

113   hairs in females. When the parasitized hosts were dead before wasps emerged, the hosts were dissected to

114   count immature parasitoids as a measure of brood size, but their sexes were not identifiable.

115   **Data Analyses**

116   General linear mixed-effects models (LMMs) were used to analyze brood size (number of wasps emerged),

117   which was transformed by logarithm before the analysis to satisfy the underlying assumptions. Generalized

118   linear mixed-effects models (GLMMs) were used to analyze sex ratio (binary variable) with a binomial error.

119   The subject wasp was included in the model as a random effects variable to control for the correlation between

120   three oviposition bouts of each wasp. Host body weight, host instar (nominal), wasp size, the temporal order of

121   oviposition (ordinal), and their second-way interactions were firstly included as fixed effects variables in the

122   full model, then excluded from it when they did not have significant effects, using Type III Wald Chi-Square

123   test to evaluate the significance of the terms by comparing the fit of full model to the fit of the reduced model

124   without each of them. Orthogonal polynomial contrasts were used to detect trends (either linear or quadratic) in

125   parasitoid performance with increasing bouts of oviposition. When there was a significant interaction between

126   host instar and body weight, general linear models were separately used to detect the relationship between

127   brood size and host body weight for each of host instars examined. To analyze the relationship between the

128   number of males in a brood and brood size, the tree model was used to determine the threshold for splitting the

129   brood size, of which the number of males in a brood changed as a step function. The mixed effects models

130   were fitted by the laplace approximation using '**lme4**' version 1.1-10 (Bates et al, 2015). Data analyses were

131   performed with R (R Development Core Team, 2014).

132

133   **RESULTS**

134   Parasitoid brood size was affected by host body weight and instar interaction (Wald Chi-Square test, χ^2 =

135   6.30, $P = 0.04$) and the order of oviposition ($\chi^2 = 18.01$, $P < 0.001$), but not by wasp HTL ($\chi^2 = 0.01$, $P = 0.94$).

136   The regression analysis of brood size in relation to host body weight for different host instars showed no

137   significant relations for the 2nd ($\chi^2 = 2.09$, $P = 0.14$) and 3rd ($\chi^2 = 0.90$, $P = 0.34$) instars. Brood size ranged

138   from 4 to 21 wasps with an average of 9.0 $\pm$ 0.3 ($\pm$ SE) in the 2nd instar hosts that varied in body weight from

139   0.01 to 0.03 mg, and 2 to 19 with an average of 9.1 $\pm$ 0.2 ($\pm$ SE) in the 3rd instar hosts that varied in body

140   weight from 0.03 to 0.14 mg (Fig. 1A, B). But the analysis showed a marginally significant relation between

141   brood size and body weight of the 4th instar hosts ($\chi^2 = 3.65$, $P = 0.048$), with a gradual increase by ca 20 % in

142   brood size with each increase of 0.1 mg in host body weight; brood size ranged from 4 to 14 wasps with an

143   average of 9.3 $\pm$ 0.3 ($\pm$ SE) across host body weight from 0.18 to 0.69 mg (Fig. 1C). Brood size did not

144   change with ovipositing wasp HTL (Fig 1D), but varied with the temporal sequence of oviposition, decreasing

145   on a linear trend over increasing bouts of oviposition (slope = $-$ 0.11, SE = 0.03, t = 4.24) from 10 offspring

146   adults emerged per host in the first oviposition bout down to 8 in the third (Fig. 1E).

147   Offspring sex ratio was not affected by host instar (Wald Chi-Square test, χ^2 = 0.89, P = 0.64), host body

148   weight (χ^2 = 0.16, P = 0.69), wasp HTL (χ^2 = 0.01, P = 0.93) and the temporal sequence of oviposition (χ^2 =

149   3.69, P = 0.16). Average proportions of male offspring from attacking host larvae of different instars were 0.14

150   (SD = 0.10) for the 2nd and 0.14 (SD = 0.11) the 3rd instar larvae, with two extremely high sex ratios (male

151   proportion > 0.5) for each of the two instars; the mean proportion of males was 0.13 (SD = 0.05) for the 4th

152   instar hosts (Fig. 2A). Male proportion of offspring did not change with host body weight (Fig. 2B) and

153   ovipositing wasp HTL (Fig. 2C), with a few extremely high sex ratios produced from very small hosts (body

154   weight < 0.1 mg) or wasps (body length < 0.45 mm). Proportions of males for successive bouts of oviposition

155   were 0.13 (SD = 0.09) for the 1st, 0.13 (SD = 0.06) the 2nd, and 0.16 (SD = 0.11) the 3rd, with two much higher

156   sex ratios produced from the 1st and 3rd bouts of oviposition, respectively (Fig. 2D). The number of males per

157   brood changed as a step function of brood size, which split at 12 with a significant difference between the two

158   split groups ($F_{1,\ 232}$ = 68.17, P < 0.001). A brood fewer than 12 contained 1 male (mean = 1.2, SD = 0.49),

159   while brood more than 12 had 2 males (mean = 1.68, SD = 2.24) with a few broods containing extremely

160   numerous males (Fig. 2E).

161

162   **DISCUSSION**

163   In this study, we showed that *O. sokolowskii* offspring brood size tended to increase with body size of *P.*

164   *xylostella* 4th instar larvae, but did not change with that of the 2nd and 3rd instar larvae. The pattern exhibited in

165   the 4th instar larval hosts is similar to that shown in prepupal hosts documented by Nakamura & Noda (2002).

166   We therefore assume that *O. sokolowskii* females may take the cues associated with body size of only older

167   larval hosts when evaluating host quality to make the oviposition decision on clutch size. Host larvae close to

168   pupation can provide more accurate information about host quality in terms of body size than younger ones do

169   because body size changes much less with growth in mature than younger larvae. Such evaluation of host

170   quality can be advantageous for koinobiont parasitoids to attack older larval hosts, such as larval-pupal

171   parasitoids. Female *O. sokolowskii* prefers to parasitize older than younger *P. xylostella* larvae (including

172   prepupae) (Talekar & Hu, 1996). Though differential mortality rates during offspring development among

173   different host instars may somehow potentially contribute to the pattern we observed in *O. sokolowskii*, such

174   effect might be negligible because brood sizes were quite close to each other between host instars observed in

175   our study, while the number of eggs (primary clutch size) are not different among these instars (Nakamura &

176   Noda, 2002).

177   Body size is one of important attributes for female wasps because it is correlated with several components of

178   the fitness (King, 1987; Blackburn, 1991; King, 1993; Ellers & Jervis, 2003; Henry, Ma & Roitberg, 2009). In

179   the case of gregarious parasitoids, the fitness of a female parasitoid can be strongly correlated with not only the

180   number of eggs laid per host but also the number of hosts attacked. Our study showed that *O. sokolowskii*

181   brood size did not change with body size of ovipositing wasps. We therefore assume that larger *O. sokolowskii*

182   females may realize their greater potential in reproduction by attacking more hosts instead of laying more eggs

183   per host. Such strategy of clutch size allocation can be adaptive to exploiting clumped distribution of hosts. As

184 the only host of *O. sokolowskii*, *P. xylostella* larvae tend to clump together in the early stage, disperse locally

185 as they grow, and then aggregate again during pupation (Chua & Lim, 1979; Furlong, Wright & Dosdall,

186 2013).

187 For the performance of a slight, linear decrease in brood size across successive bouts of oviposition shown

188 in our study, the most likely explanation is that egg expenditure on successive bouts of oviposition leads to a

189 steady decline in number of eggs available to oviposit when a group of hosts are encountered in quick

190 succession. Daily decrease in offspring production occurs in *O. sokolowskii* (Wang et al., 1999; Li, Niu & Liu,

191 2014), as well as in other gregarious parasitoids, such as in *Cotesia glomeratus* L. attacking the white butterfly

192 *Pieris rapae* (L.) (Ikawa & Suzuki, 1982).

193 Our study did not show the influence of host size, host life stage, wasp body size and oviposition sequence

194 on parasitoid offspring sex ratio. Invariable sex ratios in response to host age and size were previously

195 observed in *O. sokolowskii* (Wang et al., 1999; Nakamura & Noda, 2002; Silva-Torres et al., 2009). The lack

196 of sex allocation response to changes in host size in *O. sokolowskii* may exclude widely held Charnov's host

197 quality hypothesis as an explanation. The Charnov's hypothesis (Charnov et al., 1981) was developed

198 specifically for solitary parasitoids, in which offspring body size is strongly correlated with host body size.

199 Host size for many idiobiontic solitary parasitoids is thought to more strongly influence female than male

200 fitness (Werren, 1984; Charnov & Skinner, 1985; King, 1993). In the case of gregarious parasitoids, however,

201 the fitness of the adult wasp is determined primarily by the clutch size and possibly the sex composition

202 (Griffiths & Godfray, 1988; Hardy, Griffiths & Godfray, 1992; Godfray, 1994; Ode & Heinz, 2002).

203 Maintaining constant, female-biased sex ratios in response to variation in host quality and wasp traits in *O.*

204 *sokolowskii* suggests that this parasitoid may be able to adjust offspring sex ratios. Female-biased sex ratios are

205 most frequently exhibited in gregarious parasitoids, where there is a high probability the mating occurs

206 between siblings and that brothers compete together for mates (Godfray, 1994; Ode and Hunter, 2002; Ode and

207 Hardy, 2008). Such observations can be explained by Hamilton's (1967) theory of local mate competition

208 (LMC), which predicts that a single female should lay only enough males to fertilize all her daughters. In the

209 case of *O. sokolowskii* where sib-mating is common among offspring from a host, foundresses are expected

210 under LMC to adjust the number of males in response to brood size. Our study showed that *O. sokolowskii*

211 produced 1 male in most of broods under 12 wasps, and 2 males in broods over 12, except a few largest broods

212 with extremely numerous males from attacking very small host larvae. We suspect that these few broods were

213 consequences of constraints under non-choice circumstances in the experiments. When these extreme data

214 points are excluded, our study is the same as Nakamura & Noda (2002) in finding that only 1 male was

215 produced when the brood contained fewer than 9 offspring and 2 to 4 males when it contained more than 9

216 offspring.

217

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221

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Figure 1

Number of wasps emerged per host (brood size) in relation to host quality and wasp traits.

Fig. 1 Numbers of offspring parasitoids per host (brood size) in relation to host body weight of the 2nd (A), 3rd (B) and 4th (C) instar larvae at oviposition, wasp body length (D), and the order of oviposition (E). The solid line in panel C represents a linear relationship between brood size and host body weight with intercept 8.09 and slope 3.33, with a 95% confidence region (grey shade). The line in panel E represents a linear trend.
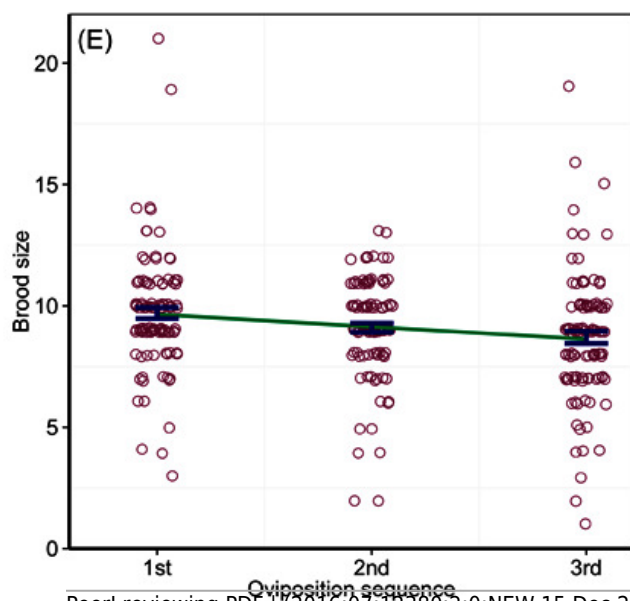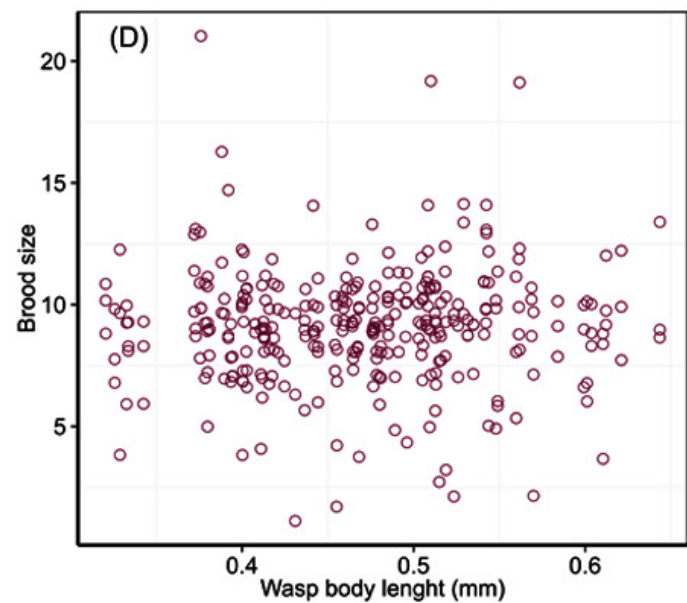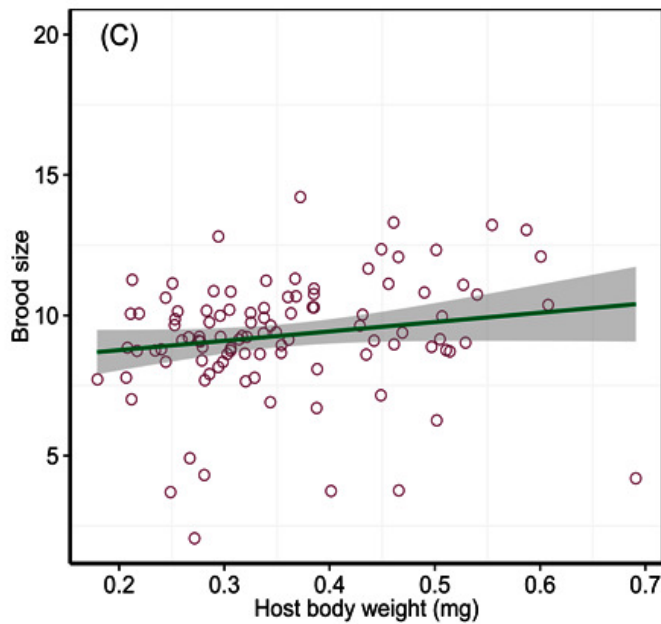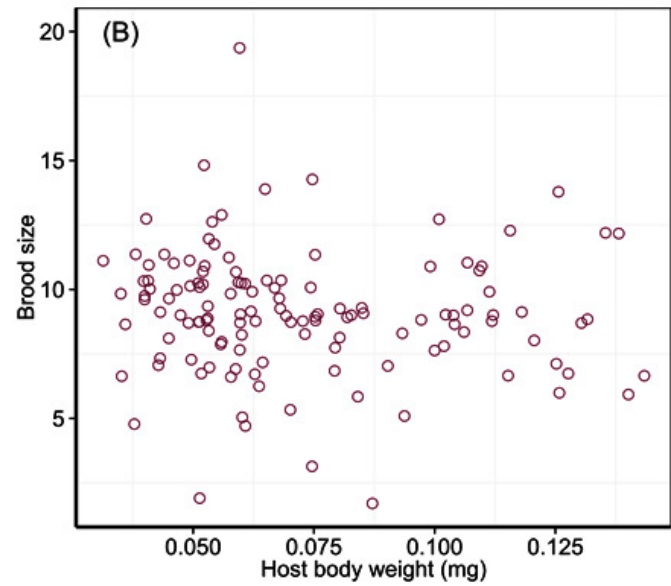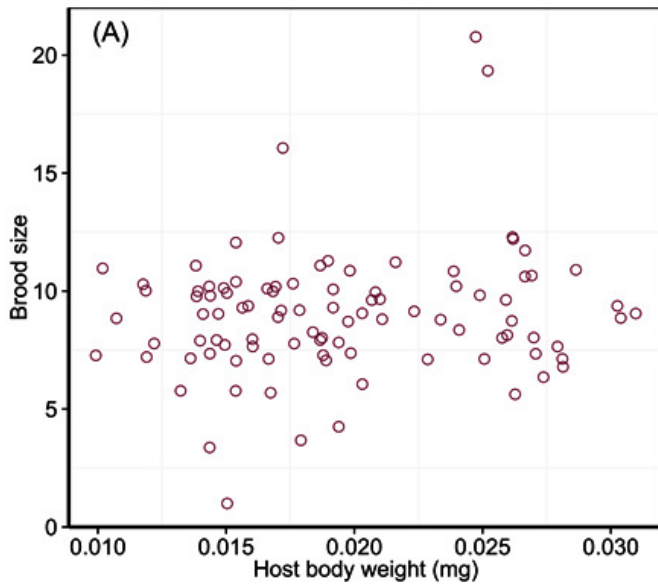
Figure 2

Brood sex ratio in response to host quality and wasp traits.

Fig. 2 Sex ratios in relation to host instar (A), host body weight (B), wasp body length (C) and bout of oviposition (D), and the relationship (E) between the number of males and offspring emerged (brood size) per host with a curve fitted by locally weighted polynomial method with a 95 % confidence region (grey shade). Bars in panels A and D are mean ± SE.