Size does not matter: No effect of signal detection and signal emission organ size on the mating success of a synchronous firefly

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The size of the organs responsible of emitting and detecting sexual communication signals is affected by sexual and natural selection. Communication via bioluminescent signals in fireflies is an interesting model to test hypotheses regarding the effect of the size of signal emission and detection organs on reproductive success. We studied the synchronous firefly *Photinus palaciosi*, a species that during the mating season congregates in large numbers in forests of central Mexico, offering a magnificent natural show that attracts numerous tourists. *P. palaciosi* operational sex ratios are strongly male-biased and the females are brachypterous. Our field study tested the hypothesis that the operational sex ratios and short daily mating period result in strong male-male competition that selects for males with larger signal detection (eyes) and signal emission (lantern) organs, whereas female-female mate competition is absent and, thus, no selection on body and lantern size is expected. Supporting the hypothesis, we found that (a) lantern size was more than four times larger in males than in females, that (b) the size of lanterns, eyes and body in females has no effect on mating success and (c) an absence of assortative mating in the size of signal detection and signal emission organs or in body size. However, contrary to our predictions, we found that (d) females have larger eyes than males and that (e) the size of lanterns, eyes and body has no effect on male mating success. Discrepancies with our predictions could be due to trade-offs with pressures different from sexual selection, such as those imposed by predators, although larger eye size in females also could be a result of intersexual selection (female choice).

1 **Size does not matter: No effect of signal detection and signal**

2 **emission organ size on the mating success of a synchronous firefly**

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16 ABSTRACT

17 The size of the organs responsible of emitting and detecting sexual communication signals is

18 affected by sexual and natural selection. Communication via bioluminescent signals in fireflies is

19 an interesting model to test hypotheses regarding the effect of the size of signal emission and

20 detection organs on reproductive success. We studied the synchronous firefly *Photinus palaciosi*,

21 a species that during the mating season congregates in large numbers in forests of central

22 Mexico, offering a magnificent natural show that attracts numerous tourists. *P. palaciosi*

23 operational sex ratios are strongly male-biased and the females are brachypterous. Our field

24 study tested the hypothesis that the operational sex ratios and short daily mating period result in

25 strong male-male competition that selects for males with larger signal detection (eyes) and signal

26 emission (lantern) organs, whereas female-female mate competition is absent and, thus, no

27 selection on body and lantern size is expected. Supporting the hypothesis, we found that (a)

28 lantern size was more than four times larger in males than in females, that (b) the size of lanterns,

29 eyes and body in females has no effect on mating success and (c) an absence of assortative

30 mating in the size of signal detection and signal emission organs or in body size. However,

31 contrary to our predictions, we found that (d) females have larger eyes than males and that (e) the

32 size of lanterns, eyes and body has no effect on male mating success. Discrepancies with our

33 predictions could be due to trade-offs with pressures different from sexual selection, such as

34 those imposed by predators, although larger eye size in females also could be a result of

35 intersexual selection (female choice).

36

37 INTRODUCTION

38 Communication between males and females is a fundamental element of the mating biology of

39 most animals (*Darwin, 1871*; *Maynard Smith & Harper 2003*; *Rosenthal, 2017*). There is a great

40 variety of organs, newly evolved or specialized via sexual selection, for the emission and

41 reception of sexual signals (*Darwin, 1871*; *Rosenthal, 2017*; *Elgar et al., 2019*). A fascinating

42 example of sexual communication involving vision is that of nocturnal fireflies (*Lloyd, 1979*;

43 *Lewis, 2016*). In these insects, adults possess an organ specialized for the emission of light

44 known as lantern. Typically, males fly searching for females, emitting flashing patterns that are

45 species- and sex-specific and that are involved in mate choice, while females emit glows or

46 flashes in response (*Lloyd, 1979*; *Lewis & Cratsley, 2008; Lewis, 2016*; *Stanger-Hall et al.,*

47 *2018*). If a successful dialogue is established, the male alights, contacts the female and a close-

48 range courtship ensues (*Lewis & Cratsley, 2008*; *Stanger-Hall et al., 2018*). It is reasonable to

49 propose that the sizes of the lantern and of the eyes are among the traits influencing the

50 efficiency of sexual communication in fireflies (*Vencl & Carlson, 1998*; *Crastley & Lewis, 2003,*

51 *2005*; *Demary et al., 2006*; *Lau & Meyer-Rochow, 2006*). Somewhat surprisingly, studies of the

52 effect of signal-emission organ size on mating success in fireflies are scant and their results are

53 inconsistent. While some studies detected an effect of male lantern size on mating success and

54 female responses in two *Photinus* species (*Vencl & Carlson, 1998*; *Crastley & Lewis, 2003,*

55 *2005*), another study found that female mating decisions in a third *Photinus* species are not

56 influenced by lantern size (*Demary et al., 2006*). The effect of signal-detection organ (eyes) size

57 on the fitness of both sexes, and of signal emission organ size on female fitness components have

58 not been studied in fireflies (but see *Crastley & Lewis, 2005*).

59   Synchronous fireflies are good subjects to study the effects on mating success of the size

60 of the organs involved in sexual communication because in these species the density of signaling

61 males is very large and the operational sex ratio is male biased, resulting in intense competition

62 between males and ample opportunities for female choice (*Lloyd, 1979*; *Lewis, 2016*), two types

63 of selective pressures that could favor larger organs sizes. In this paper, we present the results of

64 a field study of the effect of signal emission (lantern) and signal detection (eyes) organ size on

65 the probability of being found in copula, in males and females of the synchronous Mexican

66 firefly *Photinus palaciosi* (*Zaragoza-Caballero, 2012; Zaragoza-Caballero et al., 2020*). *P.*

67 *palaciosi* lives in pine-oak-fir forests of central Mexico, in the states of Estado de México,

68 Puebla and Tlaxcala, and its reproductive season goes from June to the beginning of August.

69 Mate searching, courtship and mating occur during approximately ninety minutes every night

70 (heavy rainfall prevents mating activity), starting around 20:30 h. In the study site, the

71 municipality of Nanacamilpa de Mariano Arista (Tlaxcala), thousands of males congregate under

72 the canopy of the forest during this period, flying in search for females and frequently

73 synchronizing their flashing and providing a magnificent show that attracts numerous tourists

74 (*Acle Mena et al., 2018*). The females cannot fly because they are brachypterous (i.e. their wings

75 are extremely reduced) (*Zaragoza-Caballero, 2012*), and they remain stationary in herbs at

76 heights < 60 cm and glow infrequently during the mating period. The number of sexually

77 receptive females every night is much smaller than that of males and thus the operational sex

78 ratio (OSR) is male biased (personal observation).

79   In this research we aimed to test predictions derived from the following hypothesis:

80 Given that the operational sex ratios is male biased and the daily mating period is short, male-

81 male competition for mates is intense in *P. palaciosi* and, thus, selection favors males with larger

82 eyes that could improve the ability to detect the glows of the relatively scarce females, as well as

83 males with larger lanterns that increase signal emission capabilities. Larger eye size in females

84 could be advantageous if increases the ability to detect high quality males, but considering that

85 they are the limiting sex and have large numbers of potential mates every night, it would not

86 have an effect on their probability of mating. In contrast to males, females are selected to emit a

87 glow of just enough intensity to be perceived by the males, thus no selection for increased

88 lantern size is predicted. In fact, it is even possible that females are selected to produce less

89 intense glows not only to reduce emission costs, but as a female choice mechanism to increase

90 the probability of being detected by males that are particularly good at detecting females, an

91 ability they could inherit to their male offspring. We tested the following predictions of this

92 hypothesis: (1) There is male biased sexual dimorphism in lantern size. (2) Males with larger

93 eyes and lanterns have higher probability of mating, whereas female differences in the size of

94 eyes and lanterns have no effect on their mating probability. (3) Larger males have a higher

95 probability of mating as a consequence of correlative selection (for example, due to direct

96 selection on the correlated lantern size) or direct selection (for example, if larger male size is

97 advantageous in direct competition with other males and in female mate choice; *Thornhill &*

98 *Alcock, 1983*); in contrast, females differences in body size have no effect on their probability of

99 mating. Finally, (4) the lack of selection in favor of male mate choice, due to the highly

100 competitive conditions experienced by male synchronous fireflies, results in the absence of

101 assortative mating in the size of signal detection and signal emission organs or in body size.

102 **MATERIALS AND METHODS**

103 **Sample collection**

104 Samples of males and females found in copula or solitary were collected simultaneously by a

105 team of three researchers during the daily mating period (20:30 – 22:00 h) in the middle of the

106 2016 reproduction season (between June 27 and July 15). Signalling males were collected with

107   an entomological net and solitary females and mating couples by hand. Individual mating

108   couples and solitary individuals were kept in Eppendorf vials with absolute alcohol. Captures

109   were made in seventeen places (one per night) in the municipality of Nanacamilpa de Mariano

110   Arista, Tlaxcala state, México (Appendix 1). Our collection was made under the SEMARNAT

111   (Mexican Government) permit SGPA/DGVS/06292/16.

112   **Measurement of phenotypic traits**

113   We obtained three photographs of each firefly (dorsal view, ventral view and a close up of the

114   eyes) with a digital camera (Canon™ model T3i) mounted on a disection microscope

115   (Olympus™ model SZH10). The phenotypic measurements were taken with the NIH ImageJ

116   open access software (National Institutes of Health USA, http://rsb.info.nih.gov.ij/). We

117   estimated lantern size by measuring the area covered by the lantern in the ventral-view

118   photographs. Eye size was estimated as the difference between maximum eyespan and

119   interocular space (*i.e.* approximately the sum of the maximum diameter of both eyes) in the eyes

120   close-up photographs. Body size was estimated, in the dorsal view photographs, as the area

121   covered by the elytra of the males, while in females was estimated as the area that would have

122   been covered by the elytra, had they not been reduced. The area covered by the reduced female

123   elytra was also measured to document quantitatively brachyptery.

124   **Statistical analyses**

125   Since all our predictions implied the comparison of morphological traits between two groups (all

126   males *vs*. all females for predictions 1 and 4, and males [or females] found in copula *vs*. males

127   [or females] found alone for predictions 2 and 3), we first calculated t tests for each

128   morphological trait as an exploratory measure. Because most morphological variables were

129   significantly correlated (Table 1) in the whole sample (Table 1A), as well as in females and

130   males separately (Tables 1B and 1C), we used principal component (PC) analysis to summarize

131   morphological variation. We then tested sexual dimorphism in morphological measurements

132   (Prediction 1) using a binomial generalized linear model with a logit link function, with the sex

133   of the individual as the binary response variable (0=male, 1=female) and the first two principal

134   components scores (PC1 and PC2) as explanatory variables. We evaluated separately for each

135   sex the effect of the size of morphological traits on the probability of mating success (probability

136   that an individual would be captured while copulating rather than alone) (Predictions 2 and 3).

137   We constructed a separate binomial generalized linear model with logit link for each sex, using

138   mating status (0=captured alone, 1=captured mating) as the binary response variable and PC1

139   and PC2 scores as the explanatory variables. We simplified the models using backwards stepwise

140   simplification, removing each explanatory variable in order of increasing significance and testing

141   the effect of removing that variable with a chi-squared likelihood ratio test until only terms

142   whose removal leads to worsening of the model remained (*Crawley, 2013*). We carried out these

143   analyses in R software, version 3.5.3 (*R Core Team, 2019*) using the RStudio interface (*R Studio*

144   *Team, 2016*). We tested assortative mating by calculating correlations between morphological

145   trait of males and females found in copula.

146   **RESULTS**

147   **General observations**

148   We sampled 114 females (63 solitary and 51 in copula) and 156 males (105 solitary and 51 in

149   copula). Two copulating females were not included in the analyses because the posture they had

150   after fixation in alcohol prevented obtaining correct measurements.

151   **Sexual dimorphism**

152 Separate variance t tests showed that the area covered by the lantern, as expected from Prediction

153 1, was more than four times larger in males than in females (Table 2). Body size was also larger

154 (1.5x) in males, but eye size was smaller (Table 2). As expected in a species with brachypterous

155 females, the area covered by elytra and hindwings was strongly reduced in females to about one

156 sixth of that of males (Table 2).

157   The first two PC explained 94% of the whole variance (Table 3). Factor loadings

158 indicated that PC1 was mainly influenced by body size and lantern size, while PC2 was mainly

159 influenced by eye size (Table 3). Results of the binomial generalized linear model comparing

160 females and males are presented in Table 4A. There is sexual dimorphism in PC1, a difference

161 associated to the fact that males have larger body and, as expected from Prediction 1, larger

162 lantern size than females (Table 2). Females and males are clearly separated along PC1, even

163 though there is considerable variation within sexes (Table 2; Fig. 1).

164 **Effect of phenotypic traits on mating success**

165 We measured the effect of phenotypic traits on the probability of being collected in copula

166 separately for males and females. We compared 63 females collected alone with 49 females

167 collected in copula, and 105 males collected alone with 51 males collected in copula. The t tests

168 indicated that none of the morphological traits measured differed significantly between females

169 collected in copula or alone (Table 5A) or between males collected in copula or alone (Table

170 5B). These observations were confirmed by the results of the two independent binomial

171 generalized linear models comparing females collected in copula with females collected alone

172 (Table 4B) and males collected in copula with males collected alone (Table 4C). None of the

173 female and male traits summarized by PC1 and PC2 affected the probability of being collected in

174 copula (Table 4B and 4C; Figs. 2A y 2B). Thus, our results provide no support to our

175 expectations of a higher probability of mating for males with larger eyes and lanterns (Prediction

176 2) and larger body size (Prediction 3), although are consistent with the expectations of no-effect

177 in the case of females.

178 **Assortative mating**

179 Consistent with Prediction 4, we found no evidence of assortative mating in any of the three

180 morphological traits measured (Table 6).

181

182 **DISCUSSION**

183 In this paper we tested the hypothesis that in synchronous fireflies the male biased operational

184 sex ratios and the short daily mating period result in strong male-male competition that selects

185 for males with larger signal detection (eyes) and signal emission (lantern) organs, as well as

186 larger body sizes. On the other hand, in these fireflies female-female competition for mates is

187 apparently absent, and thus no selection on body and lantern size is expected in females,

188 although intersexual selection (female choice) could favor females with larger signal detection

189 organs (eyes).

190   In agreement with our hypothesis, males of *P. palaciosi* have larger lanterns (Prediction

191 1; Table 4A and Figure 1) and larger body sizes than females (Table 4A and Figure 1). Thus, our

192 data support the idea that strong intrasexual selection in males causes sexual dimorphism in

193 lantern and body size. On the other side, the observation that females evolved larger eyes than

194 males suggests that also intersexual selection (female choice) could affect sexual dimorphism,

195 although we do not know if larger eyes evolved to improve mate detection and assessment or if

196 other pressures, such as predator detection, were also involved (see below). Anyway, this last

197 result is intriguing because males of other *Photinus* species have larger eyes than females, a fact

198 considered evidence of selection favoring males with improved ability to detect females (*Lloyd,*

199 *1966*; *Demary et al., 2006*).

200   We did not find evidence for an effect on mating success of any of the morphological

201 traits measured, either in females or males (Tables 4B and 4C and Figure 2). Thus, there is no

202 evidence of an advantage for males with larger lanterns and eyes (Prediction 2) or body size

203 (Prediction 3). A expected (Prediction 4), we did not detect assortative mating in any of the

204 phenotypic traits studied. Perhaps the best species to compare our results is *P. pyralis* (*Vencl &*

205 *Carlson, 1998*), a species resembling *P. palaciosi* in that there is "intense competitiveness:

206 aggregations of males regularly attain very high densities", sometimes resulting in several males

207 attempting to mate with the same female (*Vencl & Carlson, 1998*), as we have observed in *P.*

208 *palaciosi* (personal observations). In contrast to our findings, in *P. pyralis* the body size (elytral

209 length) and lantern area of males affected the probability of being found in copula. Interestingly,

210 in this species larger males and males with larger lanterns were more successful when single

211 males courted females (the most common case: 70% of all matings), however smaller males had

212 an advantage when four or more males simultaneously courted a female "on foot" on her perch

213 (12% of all matings). According to the authors, these contrasting effects "obscured" the global

214 effect of elytral and lantern length on male mating success (*Vencl & Carlson, 1998*). When we

215 collected many of the copulating pairs there was at least one additional male close to the

216 copulating pair, unfortunately we did not make a record of this fact, thus a trade-off similar to

217 that proposed by Vencl & Carlson (1998) is conceivable and could explain the lack of effects of

218 morphological measures on male mating success.

219   Our results, on the other hand, are similar to those obtained in the non-synchronous *P.*

220   *greeni*, where the size of lanterns, eyes and body had no effect on the probability of males being

221   found alone or in copula (*Demary et al., 2006*). In this species, as well as in other *Photinus*

222   species (*Branham & Greenfield, 1996*; *Crastley & Lewis, 2003*; *Demary et al., 2006*; *Lewis,*

223   *2016*), elements of the flashing pattern are important in determining male mating success.

224   However, elements of the flashing pattern are also important in *Photinus ignites*, a non-

225   synchronous firefly in which also a significant effect of lantern size and body size on mating

226   success has been observed (*Crastley & Lewis, 2003, 2005*). A study of the effect of the flashing

227   pattern, and its possible interaction with lantern size, on the mating success of males and females

228   would be very interesting in *P. paaciosi*.

229   In the specific case of the signal detection organs (eyes), we did not find an effect of eye

230   size on male or female mating success. This fact could be explained if other selective pressures

231   also affect eye size. The morphology, physiology and behavior of signal detection and emission

232   organs are frequently influenced by selective pressures not related to the sexual communication

233   function (*Niven & Laghling, 2008*; *Stöckl et al., 2013*; *Elgar et al., 2019*). For example, the

234   detection and assessment of visual signals of mate quality (*Lewis, 2016*; *Rosenthal, 2017*; *Elgar*

235   *et al., 2019*; *Stanger-Hall et al., 2018*) suggests that the structure and function of the eyes has

236   evolved influenced by intersexual selection (mate choice). However, the eyes are also used to

237   detect other resources (food, shelter, etc.) or natural enemies and, thus, its evolution is also

238   affected by natural selection (*Elgar et al., 2019*). In *P. palaciosi*, we have detected several

239   predators of males (e.g., orb-webbing spiders and grasshoppers) during the mating period that

240   could be a significant selective pressure (personal observations). Interestingly, eye size was

241   smaller in males than in females, suggesting that in females selection pressures derived from, for

242   example, processes such as female choice, predator avoidance, prey searching (which usually

243   occurs in the ground; Lewis, 2016) and the choice of perch for mate location, have been (and

244   are) important to understand the evolution of eye size.

245   In contrast to the eyes, the lantern originally evolved as a sexual signal-emission organ,

246   which subsequently could have been affected also by natural selection (*Branham & Wenzel,*

247   *2003*; *Woods et al., 2007*; *Lewis & Cratsley, 2008*; *Stanger-Hall et al., 2018*). For example, a

248   study of two *Photinus* species determined that flashing increases dramatically predation risk and

249   increases the metabolic rate 37% with respect to the basal metabolic rate (even though the

250   experimental setting excluded flight) (*Woods et al., 2007*). In *P. palaciosi* it is not known if some

251   predator exerts a similar pressure on signaling fireflies and if this possible effect is, in turn,

252   affected by lantern size. For orb-webbing spiders—predators of *P. palaciosi* that are relatively

253   abundant in the study area—light emission seems irrelevant.

254   Firefly populations worldwide are declining and threatening factors vary in importance

255   for different species and regions (*Lewis et al., 2020*). Light pollution and tourism are considered

256   important threats for the charismatic synchronous species, such as *P. palaciosi,* and these factors

257   have their main impact during the mating period. Unfortunately, the mating system of only a

258   handful of species (out of the about 2,000 described) has been studied. Conservation measures

259   and regulation of touristic activities in fireflies "sanctuaries" need to be based on solid scientific

260   information.

261

262   **CONCLUSIONS**

263 Communication during sexual interactions requires signal emission and signal detection organs.

264 Sexual selection and other selection pressures affect the size of these organs. Our study of the

265 size of signal emission (lanterns) and signal detection (eyes) organs in the synchronous firefly *P.*

266 *palaciosi* tested the hypothesis that the male-biased operational sex ratios and the short nightly

267 mating period result in strong male-male competition, that selects for males with larger eyes and

268 lanterns, in contrast to females where mate competition is absent. In support of the hypothesis,

269 we found that lantern size was more than four times larger in males than in females, that in

270 females the size of lanterns, eyes and body has no effect on mating success, and an absence of

271 assortative mating for any of these traits. On the other hand, contrary to our predictions, we

272 found that in males the size of lanterns, eyes and body has no effect on their mating success. In

273 contrast to other fireflies, in *P. palaciosi* females have larger eyes than males. Discrepancies with

274 our predictions could be due to trade-offs with pressures different from sexual selection, such as

275 those imposed by predators, although larger eye size in females also could be a result of

276 intersexual selection (female choice).

277

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283

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Morphological correlation matrices

Morphological correlation matrices for (A) males and females together, (B) females and (C) males of the firefly *Photinus palaciosi*. In males the area covered by elytra was used as a measure of Body size; since females have reduced wings, Body size was estimated as the area of the body that in males is covered by the elytra. Significant correlations are in italics.

A) Males + Females	Lantern (mm²)	Eyes (mm)	Body size (mm²)
Eyes (mm)	*r = -0.43* *P = 1.53 x 10⁻¹³*	1	
Body size (mm²)	*r = 0.84* *P = < 2.2 x 10⁻¹⁶*	*r = -0.29* *P = 1.71 x 10⁻⁶*	1
Elytra (mm²)	*r = 0.97* *P = < 2.2 x 10⁻¹⁶*	*r = -0.44* *P = 3.22 x 10⁻¹⁴*	*r = 0.85* *P = < 2.2 x 10⁻¹⁶*
B) Females			
Eyes (mm)	r = 0.03 P = 0.77	1	
Body size (mm²)	*r = 0.60* *P = 2.38 x 10⁻¹²*	r = 0.05 P = 0.59	1
Elytra (mm²)	*r = 0.59* *P = 7.73 x 10⁻¹²*	r = -.0003 P = 0.99	*r = 0.76* *P = < 2.2 x 10⁻¹⁶*
C) Males			
Eyes (mm)	r = 0.01 P = 0.90	1	
Body size (mm²)	*r = 0.85* *P = < 2.2 x 10⁻¹⁶*	r = 0.04 P = 0.62	1

Statistical summary and comparison of morphological measures of males and females

Comparison of the morphological measurements of male and female *Photinus palaciosi* fireflies. Average ($Q_{25\%}$–$Q_{75\%}$) [minimum value–maximum value] are given for each trait. Results of independent variances t tests are given in the last column as follows: P value (t value) [degrees of freedom]. * The body size of males was estimated with the area covered by the elytra.

	Females	Males	t test
Lantern (mm^2)	0.79 (0.66–0.91) [0.41–2.04]	3.54 (2.86–4.19) [1.65–6.08]	$P < 2.2$ x 10^{-16} (-38.728) [180.21]
Eyes (mm)	1.77 (1.56–1.99) [0.74–2.96]	1.32 (0.99–1.51) [0.49– 2.64]	$P < 2.2$ x 10^{-16} (9.252) [257.11]
Body size (mm^2)	20.48 (17.26–23.41) [11.34–40.69]	30.73 (25.68–35.41) [17.27–53.64] *	$P < 2.2$ x 10^{-16} (-14.641) [264.35]
Elytra (mm^2)	4.86 (4.17–5.47) [3.13–4.43]	30.73 (25.68–35.41) [17.27–53.641] *	$P < 2.2$ x 10^{-16} (-49.231) [166.32]

Principal components of morphological measurements.

Summary of principal components analysis of morphological traits of the synchronous firefly *Photinus palaciosi*. The data presented are the factor loading coefficients for each morphological trait and the percentage of variation explained by the first two PC. Sample size is 268 (112 females and 156 males).

	PC1	PC2
Lantern size (mm^2)	0.651	-0.186
Body size (mm^2)	0.612	0.445
Eye size (mm)	-0.449	-0.876
Eigenvalue	2.073	0.751
% variation	69.12	25.03

Generalized Linear Models results.

Results of models evaluating (A) sexual dimorphism (probability that an individual will be male versus female), and probability of being encountered copulating rather than alone in females (B) and males (C), in relation to the scores of the first two principal components of body measurements of the synchronous firefly *Photinus palaciosi*. Variables indicated in bold were significant, and therefore included in the minimum adequate model.

Fixed Effects	$\beta \pm SE^{*}$	Z^{*}	P_Z^{*}	$P_{\chi^2}^{\S}$
A) Sexual Dimorphism				
PC1	**1.634 ± 0.408**	**4.001**	**6.3 x 10^{-05}**	**3.112 x 10^{-05}**
PC2	**-0.510 ± 0.279**	**-1.828**	**0.068**	**0.045**
B) Female probability of mating				
PC1	-0.0893 ± 0.565	-0.158	0.874	0.06
PC2	-0.752 ± 0.513	1.466	0.143	0.874
C) Male probability of mating				
PC1	-0.219 ± 0.601	-0.363	0.716	0.714
PC2	-0.504 ±0.353	-1.426	0.154	0.131

4 **Notes:**

5 *Parameters from initial (full) model

6 $^{\S}P$-value resulting from χ^2 nested model comparisons following removal of each variable during backwards stepwise

7 simplification

Morphological measures of males and females found alone or in copula.

Comparison of the morphological measurements of (A) females found in copula vs. females found solitary, and (B) males found in copula vs. males found solitary, in *Photinus palaciosi* fireflies. Average ($Q_{25\%}$–$Q_{75\%}$) [minimum value–maximum value] are given for each trait. In the last column, results of independent variances t tests are given as follows: P value (t value) [degrees of freedom].

	Solitary	In copula	t test
A) Females			
Lantern (mm²)	0.80 (0.66–0.97)	0.78 (0.645 – 0.865)	P = 0.65 (-0.46)
	[0.53–1.25]	[0.413–2.04]	[89.34]
Eyes (mm)	1.79 (1.56–2.01)	1.75 (1.562 – 1.963)	P = 0.61 (-0.52)
	[1.05–2.71]	[0.738–2.961]	[94.70]
Body size (mm²)	20.09 (16.60– 23.11)	20.99 (17.48–21.11)	P = 0.37 (0.90)
	[11.66–32.11]	[11.36–40.69]	[84.05]
Elytra (mm²)	4.79 (4.15–5.33)	4.95 (4.20– 5.53)	P = 0.45 (0.76)
	[3.20–7.34]	[3.13–9.43]	[81.92]
B) Males			
Lantern (mm²)	3.5 (2.84– 4.15)	3.62 (2.96–4.24)	P = 0.44 (0.77) 84.32
	2.05–5.52	1.65–6.08	
Eyes (mm)	1.30 (0.97–1.5)	1.34 (1.09–1.58)	P = 0.53 (0.62) 116.44
	0.49–2.64	0.82–2.48	
Body size (mm²)	30.67 (25.89–35.25)	30.84 (25.57–36.35)	P = 0.88 (0.15) 89.38
	17.95–47.71	17.27–53.64	

Correlation matrix of males and females found in copula.

Correlation matrix of the morphological traits of males and females found in copula in *Photinus palaciosi* fireflies. No significant correlations were found (N = 49)

Females	Males		
	Lantern size (mm^2)	Body size (mm^2)	Eye size (mm)
Lantern size (mm^2)	r = 0.21	r = 0.11	r = -0.06
	P = 0.16	P = 0.45	P = 0.68
Body size (mm^2)	r = 0.09	r = 0.05	r = 0.04
	P = 0.52	P = 0.74	P = 0.78
Eye size (mm)	r = 0.05	r = 0.01	r = 0.12
	P = 0.75	P = 0.94	P = 0.41

Figure 1

Sexual dimorphism of *Photinus palaciosi.*

First two principal components summarizing the morphological traits measured in females and males of the synchronous firefly *P. palaciosi*.
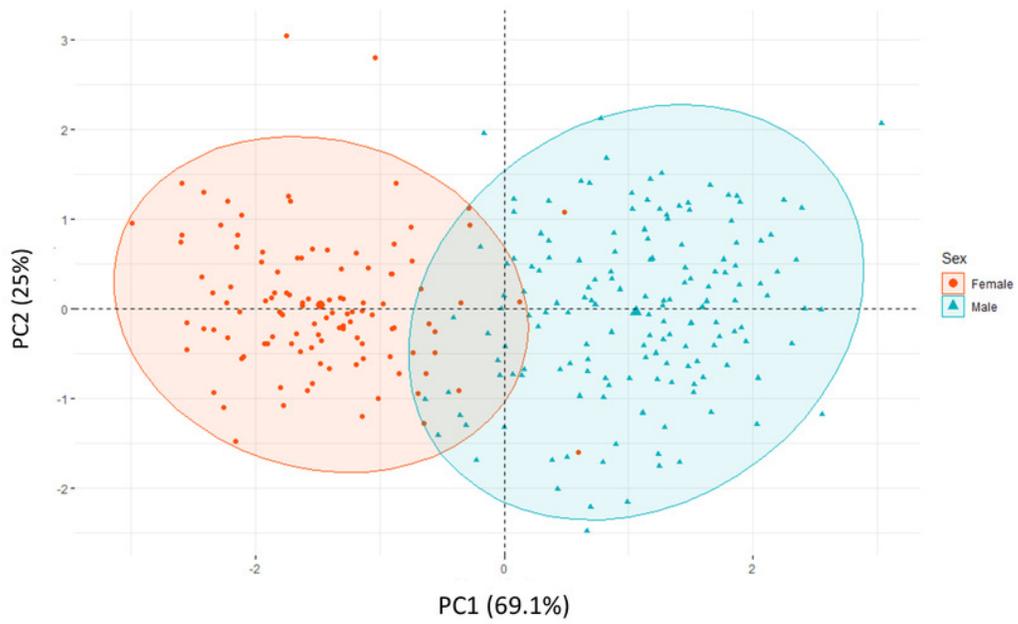
Figure 2

Comparison of morphological measurements of *P. palaciosi* fireflies collected alone or in copula.

Principal components comparison of individuals collected alone or in copula in (A) females and (B) males of the synchronous firefly *P. palaciosi*.