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2 **Title**

3 Relationships between mating tactics and male traits such as body size and
4 fluctuating asymmetry in the Japanese scorpionfly

5

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17

18 **Abstract**

19 Fluctuating asymmetry (FA) is defined as small, random deviations from perfect
20 bilateral symmetry in a morphological trait. It has sometimes been used as an
21 indicator of male quality because it affects male mating success. In the Japanese
22 scorpionfly, *Panorpa japonica*, males are known to use two mating tactics, donation
23 of nuptial gifts and forced mating. In *P. japonica*, low FA males are more likely to win
24 a competition with another male, and females prefer low FA male sex pheromones.
25 However, no studies have been conducted on the effect of FA on the mating tactics
26 adopted by males. In the present study, we first observed the mating behaviors of *P.*
27 *japonica* and classified these into three patterns: 1) nuptial gifting, 2) forced mating,
28 and 3) feeding mating. Second, we investigated the relationships between mating
29 tactics and male traits, including body size, FA, and weapon size. The results showed
30 a positive correlation between absolute FA and mating duration only in the case of
31 feeding mating. We discuss the reasons why this significant correlation was found in
32 *P. japonica*.

33

34 Key words: copulation; courtship; mating duration; *Panorpa japonica*

35

36 **Introduction**

37 Sexual selection leads to the evolution of male traits that are advantageous for
38 male-male competition and female mate choice (e.g., Andersson 1994; Miller and
39 Svensson 2014). Male body size and weapon size are related to sexual selection in
40 many species (e.g., Calder 1984 Peters 1986; Blanckenhorn 2000). In insects, larger
41 males usually have an advantage in male-male competition and thus tend to have
42 more mating opportunities with females compared with those of smaller males (e.g.,
43 Thornhill and Alcock 1983; Emlen 2008). On the other hand, smaller males avoid
44 fighting, and they use satellite or sneaking tactics instead (e.g., Arak 1988; Gross
45 1996). For example, during copulation in the Japanese stag beetle, *Prosopocoilus*
46 *inclinatus*, the duration of mounting by smaller males is longer than that by larger
47 males, and smaller males force mating more frequently than larger males (Okada
48 and Hasegawa 2005). Additionally, in some insect species, such as the Japanese
49 rhinoceros beetle, *Trypoxylus dichotomus*, and the yellow dung fly, *Scathophaga*
50 *stercoraria*, a negative correlation between body and weapon size and mating
51 duration has been reported (Ward and Simmons 1991; Karino and Niiyama 2006).

52 Fluctuating asymmetry (FA) influences sexual selection (Møller 1990). FA is
53 defined as small, random deviations from perfect bilateral symmetry in a
54 morphological trait (Møller and Pomiankowski 1993). FA is caused by low genetic
55 quality, nutritional status, and environmental stress during growth stages (Palmer
56 and Strobeck 1986; Leary and Allendorf 1989; Parsons 1990; Thornhill and Sauer,
57 1992). In the Australian sheep blowfly (*Lucilia cuprina*), FA is a very sensitive
58 indicator of environmental stresses in the larval stage because FA is strongly affected
59 by population density and temperature stress during the larval stage (Clarke and
60 McKenzie, 1992). Additionally, in various species, reproductive success is higher in

61 males with smaller FA than in males with larger FA (Møller 1988, 1990; Harvey and
62 Walsh 1993; Koshio et al. 2007).

63 For example, in males of the scorpionfly *Panorpa vulgaris*, FA may be an indicator
64 of sexual selection because bilateral forewing symmetry has a genetic basis and
65 because males with symmetrical forewings are more likely to win male-male
66 competitions (Thornhill and Sauer 1992). However, some studies have reported no
67 significant correlation between genetic quality or the presence or absence of
68 environmental stress and bilaterally symmetric fluctuations (e. g., Bjorksten et al.
69 2000, Woods et al. 1999). Early studies of FA and mating success also failed to
70 evaluate measurement errors and the reproducibility of FA measurements
71 (Simmons et al. 1999). Furthermore, a meta-analysis showed that publication bias
72 occurred in earlier studies of FA and mating success (Palmer 2000). Thus, the
73 relationship between FA and mating success in the context of sexual selection is now
74 questioned. If FA is related to sexual selection, mating tactics might also be affected
75 by FA. However, to our knowledge, there are no studies on the effects of FA on mating
76 tactics and mating duration.

77 Males of many scorpionfly (Panorpidae) species provide nuptial gifts to females
78 during mating. A nuptial gift is food or a nutritious secretion provided before or
79 during copulation (Thornhill and Alcock 1983). The nuptial tactics of male
80 scorpionflies are to attract females by sex pheromones, and then the male provide
81 food, such as arthropod carcasses or nutritious saliva secretions, to the females
82 (Sauer et al. 1997).

83 In the Japanese scorpionfly, *Panorpa japonica*, males exhibit a characteristic
84 posture presumably for releasing sex pheromone near bait and give it to an
85 approaching female as food for a nuptial gift before mating (Thornhill 1992a).

86 Thornhill (1992b) observed the behavior of male Japanese scorpionflies and
87 reported that males frequently perform male-male combat for nuptial gifts and try
88 to catch females using the graspers at the end of their abdomen. When males
89 approach females without a nuptial gift, this behavior is called “forced mating”.
90 Thornhill (1992b) also observed male-male competition and then reported that FA
91 of forewing length is significantly less in winners than in losers, while females prefer
92 the pheromone of males of relatively low FA in forewing length (Thornhill 1992a).
93 In addition, high FA males have lower lifetime mating frequencies with different
94 females than low FA males (Thornhill 1992a, b).

95 Therefore, high FA males usually choose other tactics (such as force mating) over
96 nuptial gifts. A high FA male might invest a lot in the mating duration of one
97 copulation event. However, there are no experimental studies of the relationships
98 between FA, mating tactics, and mating duration. Thus, in the present study, we
99 examined the relationships between FA and male body size, grasper (weapon used
100 for direct male-male competition) size, mating tactics, and mating duration in *P.*
101 *japonica*.

102

103

104 **Materials and Methods**

105 **Insects**

106 Forty males and 40 females of *P. japonica* were collected from Handa-yama
107 Mountain, Okayama City, Japan (latitude: 34°6′N, longitude: 133°9′E), from 1 to 15
108 May 2017 to minimize the effects of sampling time on male mating tactics. The
109 experiment was conducted for one week after collection. In addition, we investigated
110 the effects of the date and time of collection on the mating duration and mating

111 tactics of the individuals used in the experiment, but no correlation was found
112 (Wilcoxon's rank sum test $Z=0.4437$, $p>0.05$). Therefore, we consider no periodical
113 effect of insect collection on the results of this study.

114 Each adult was reared in a plastic container (10 cm diameter × 8 cm height) in a
115 chamber maintained at 25°C and 16 L: 8 D until the experiment was conducted. A
116 piece of mealworm (*Tenebrio molitor*; weight: 0.2 g; length: 5 mm) was given once
117 every two days.

118

119 **Observation**

120 A randomly chosen male and female were placed in a cylindrical plastic container
121 (10 cm diameter × 10 cm height) in which a piece of mealworm (0.2 g) had been
122 placed on the bottom with appropriately moistened absorbent cotton. After the pair
123 was settled, mating behaviors were recorded with a video camera (HDR-PJ590V,
124 Sony, Tokyo). The mating duration was defined as the period from the point at which
125 the male grabbed the female's abdomen to the point when the male released the
126 female. Each male and female pair was used for only a single observation.

127

128 **Measurement**

129 After the recording was finished, each individual was preserved in 70% ethanol,
130 and the lengths of the left and right forewings were measured using a stereoscopic
131 microscope (×7) (Olympus SZX12, Tokyo) and microscope camera (INOCAM-HD2,
132 Inohara Shokai, Hiroshima) according to the method described by Thornhill (1992a)
133 (Fig. 1). Before we measured the wing length, we dried the wings to remove any
134 moisture. In the species used in this study, the forewing anal vein was unclear (see
135 Thornhill 1992a). Therefore, here, the forewing length was measured from the

136 starting point of the anal vein to the third radial vein (see Fig.1). Thornhill (1981)
137 showed that forewing length can be an accurate index of body size in *Panorpa*
138 species. For *P. japonica*, Thornhill (1992b) defined forewing length as the entire
139 length of the anal vein. However, since its distal end was unclear in our samples, we
140 measured the length from the proximal end of the anal vein to the distal end of the
141 third radial vein in this study (Fig. 1). In this study, forewing size ($\hat{=}$ body size) refers
142 to the sum of the left and right forewing lengths divided by 2 according to the method
143 described by Thornhill (1992a).

144 FA was defined as the absolute value of the difference between the right and left
145 wings. Each measurement of the forewings was taken three times. The three
146 measurements for each forewing were used in all analyses. Grasper size was defined
147 as the length from the first protrusion counted from the inside (Fig. 2A) to the base
148 to the tip of the grasper (Fig. 2B). The grasper base size was defined as the length
149 from point A of Fig. 2 to the outside tip of the grasper base (Fig. 2C). Because the
150 difference in body size between males and females may affect mating tactics and
151 mating duration, we used the absolute values of the difference in body size between
152 males and females. The ImageJ software program (Ver. 1.50i) was used for all the
153 measurements. We adopted the absolute FA values, i.e., FA divided by body size, as
154 additional indicators of FA. Note that all measurements of FA in the present study
155 are absolute, not relative, values.

156

157 **Statistical analysis**

158 The one-way analysis of variance (ANOVA) was used to compare the mating
159 durations of the three tactics. A mixed model ANOVA with three replicate

160 measurements of body size and FA as a random factor was used to compare body
161 size and FA among the tactics. Spearman's rank correlation coefficients were used to
162 analyze the relationships between mating duration and size parameters, including
163 FA. ANCOVA was used to assess the measurement error and reproducibility of FA in
164 the relationships between mating duration and size parameters, including FA.

165 The statistical package JMP version 12.2 (SAS Institute Inc., 2015) was used for all
166 analyses.

167

168 **Results**

169 **Behavioral sequences in male mating**

170 Fig. 3 shows a behavioral sequence of male mating behavior. Three mating tactics
171 were categorized based on the following criteria: (A) nuptial gifting: males found a
172 bait, remained near the bait, released a pheromone, and succeeded in mating, (B)
173 feeding mating: males did not release pheromones and did not wait near bait but
174 approached a female who was eating bait and then succeeded in mating, and (C)
175 forced mating: males approached females without releasing pheromones while the
176 females were not eating a bait.

177 The mating patterns of forty pairs were observed. At the encounter stage, 21
178 males found bait and released pheromones, 13 males found females that were eating
179 the bait but did not release pheromones, 4 males found females that were not eating
180 bait but did not release pheromones, and 2 males released pheromones without
181 finding either a female or the bait. Of the 21 males that found bait and released
182 pheromones, 15 presented a nuptial gift to the female and then mated with the
183 female (A: nuptial gift), but the remaining 6 males did not mate. Of the 13 males that
184 found a female that was eating the bait, 12 males mated with the female (B: feeding

185 mating), but one male did not mate because he was driven away by the female. The
186 four males that found a female that was not eating the bait proceeded to force mating
187 (C: forced mating). The two males that released pheromones without finding a
188 female or the bait did not mate.

189 In the following comparisons, we used three types of mating tactics, i.e., (A) nuptial
190 gift, (B) feeding mating, and (C) forced mating, to compare mating duration, FA, male
191 body size, and grasper size.

192

193 **Relationships between mating tactics and mating duration**

194 The mean mating durations for the three mating tactics were compared (Fig. 4). No
195 significant differences were found among the three tactics (one-way ANOVA, $F_{2, 30}$
196 $=0.4303$, $p=0.6546$). In the following analyses, forced mating (C) was removed
197 because of the small sample size.

198

199 **Body size and FA of males, mating duration, and grasper size in two mating** 200 **tactics**

201 We compared the FA and body size of the males that performed (A) nuptial gifting
202 and (B) feeding mating (Fig. 5). Based on the mixed model ANOVA, which included
203 three replicates as a random factor, significant differences were found between the
204 two mating tactics for the absolute values of FA (mixed ANOVA, $F_{1, 25} = 8.3462$,
205 $p=0.0079$) (top graph of Fig. 5), although no significant differences were found
206 between the two mating tactics for body size (mixed ANOVA, $F_{1, 25} = 0.1016$,
207 $p=0.7525$) (bottom graph of Fig. 5).

208 The reproducibility of the forewing FA measurements was tested among the three
209 replications, and the reproducibility was confirmed (ANCOVA, Table 1). The

210 relationships between male body size/male FA in the three replications and mating
211 duration are shown in Table 2. In nuptial gift mating, the relationships between male
212 body size and mating duration and between FA and mating duration were not
213 significant in any of the replications. In feeding mating, a significant negative
214 correlation was found between the FA and mating duration in each replication.
215 However, no significant relationships were found between male body size and
216 mating duration in any of the replications.

217 Fig. 6 shows the male grasper size (top graph) and grasper base size (bottom
218 graph) for the nuptial gifting (A) and feeding mating (B) tactics. No significant
219 differences were found in the male grasper size (Wilcoxon signed-rank test, $p =$
220 0.4155) or grasper base size (Wilcoxon signed-rank test, $p = 0.3000$) between these
221 two tactics.

222

223 **Effect of size differences in pairs on mating duration and mating tactics**

224 For each mating tactic, there was no significant correlation between the mating
225 duration and the size difference of the pairs, male grasper size, or male grasper base
226 size (Table 3).

227

228 **Discussion**

229 **Male mating tactics**

230 Many European scorpionflies use nutritious saliva as a nuptial gift, and a positive
231 relationship between the amount of bait used in a nuptial gift and mating duration
232 was confirmed in *P. vulgaris* (Sindern 1996; Sauer et al. 1997, 1998; Sauer 2002).

233 In contrast, males of *P. japonica* use dead arthropods instead of nutritious saliva as
234 nuptial gifts (Thornhill 1992b). The mating behavior observed in this experiment is

235 similar to that of *P. liui*. Both the male and female *P. liui* have simple salivary glands;
236 therefore, it is thought that this species does not use salivary masses for copulation,
237 unlike *P. vulgaris* (Ma and Hua 2011). In *P. japonica*, as in *P. liui*, only small arthropods
238 are used for nuptial gifts because of the immaturity of the salivary glands of the
239 males.

240 In the present study, *P. japonica* females consumed bait during mating (Fig. 4). In *P.*
241 *japonica*, a female may arrive earlier than the male and eat the bait (we defined this
242 as feeding mating). In this case, the males may adopt feeding mating tactics (B)
243 rather than nuptial gift tactics (A), unlike *P. vulgaris*. Moreover, *P. japonica* males use
244 dead arthropods as nuptial gifts. This difference in mating patterns may cause a
245 different correlation between species of the same genus. It is necessary for future
246 studies to further evaluate the mating durations of many species that use nutritious
247 saliva and dead arthropods as nuptial gifts.

248

249 **Relationship between male mating tactics and male traits**

250 Thornhill (1992a, b) found no relationships between male body size and the results
251 of male-male competition or a female's preference for pheromones in *P. japonica*. He
252 also reported that males that have symmetrical forewings easily win male-male
253 competitions and that females are attracted more to the pheromones released by the
254 males who have symmetrical rather than asymmetrical forewings.

255 In this study, the males with more asymmetrical forewings were significantly more
256 likely to adopt the feeding mating tactic than the nuptial gift tactic (top graph of Fig.
257 5). On the other hand, no difference was found in the sizes of the fly bodies or
258 graspers, which are the male weapons (bottom graph of Fig. 5) between the two
259 mating tactics. This finding suggests that male mating tactics are affected by the FA

260 of the forewing, but that male body size and weapon size do not affect male mating
261 tactics (Fig. 6). In the present study, however, we did not focus on nutritional
262 conditions. In the future, it will be necessary to conduct experiments to manipulate
263 nutritional status. Also, it is necessary to examine the effects of mating experience,
264 male-male competition, and nutritional status on mating tactics in the future.

265

266 **Relationship between mating duration and male traits**

267 In the present study, no significant relationship was found between mating
268 duration and male body size or FA when nuptial gift tactics were used (Table 2).
269 When feeding mating tactics were used, a significant positive relationship was found
270 between FA and mating duration (Table 2), but no relationship was found between
271 male body size and mating duration (Table 2). These results suggest that the FA of
272 male forewings may affect mating duration when feeding mating tactics are used by
273 *P. japonica*. In males with asymmetrical forewings, the mating durations were longer
274 than those in males with symmetrical forewings.

275 High FA males have a lower lifetime mating incidence with different females than
276 low FA males (Thornhill 1992b). Therefore, high FA males may increase their own
277 mating success by increasing the mating duration. In this study, in high FA males,
278 feeding mating had a longer mating duration than that of nuptial gift mating. High
279 FA males may not be able to attract females with sex pheromones and can easily lose
280 in male-male competitions (Thornhill 1992a, b), making it difficult to mate with
281 nuptial gift tactics. Therefore, high FA males may have longer mating durations when
282 using feeding mating tactics than nuptial gift tactics. However, the relationship
283 between the mating duration and the amount of sperm transferred by *P. japonica*
284 was not investigated in the present study. To verify whether an increase in mating

285 time leads to successful mating in this species, the sperm amount in the spermatheca
286 should be measured using virgin females in further experiments.

287 Alternatively, it may also be possible that an asymmetrical male's reproductive
288 organs had “abnormal” growth, and thus, their mating durations were prolonged
289 compared with those of the symmetrical males by their malformed reproductive
290 organs. It is necessary to increase the sample size in future studies because the
291 present study used a small sample. In addition, it is important that the mating tactics
292 of *P. japonica* in the field be examined in the near future.

293

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298

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380

381 Tables
 382

Table 1. Results of the reproducibility analysis of forewing FA measurement (ANCOVA)

treat	nuptial gift		feeding mating	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
body size	0.0012	0.9988	0.0063	0.9937
FA	0.1566	0.8555	0.0595	0.9424

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Table 2. Relationships between male body size/male FA in the three replicatians and mating duration (Spearman's rank correlation coefficient).

mating tactics	treatment	replrcation1		replrcation2		replrcation3	
		ρ	<i>p</i>	ρ	<i>p</i>	ρ	<i>p</i>
nuptial gift	body size	-0.0393	0.8894	-0.0214	0.9396	-0.0214	0.9396
	FA	-0.195	0.4862	-0.1609	0.5668	-0.0841	0.7658
feeding mating	body size	-0.4545	0.1377	-0.4755	0.1182	-0.4336	0.1591
	FA	0.6783	0.0074	0.7075	0.0101	0.6643	0.0185

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Table 3. Relationships between mating duratoin and the size differences of pairs, nale grasper size, and male gasper base size in each mating tactic

mating tactics	N	the size difference of pairs	male grasper size	male grasper base size
nuptial gift	15	ρ = -0.2714	ρ = 0.1429	ρ = 0.2556
		<i>p</i> = 0.3278	<i>p</i> = 0.6115	<i>p</i> = 0.3579
feeding mating	12	ρ = -0.0559	ρ = -0.007	ρ = -0.021
		<i>p</i> = 0.8629	<i>p</i> = 0.9828	<i>p</i> = 0.9484

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391 Legends of figures

392

393 Fig. 1 Diagram of the right forewing of the species used in this experiment (*Panorpa*
394 *japonica*). We defined the distance from the starting point of the anal vein (A) to the
395 third radial vein (RV), as shown in the figure, as the length of the forewing.

396

397 Fig. 2 Diagram of the right grasper of *Panorpa japonica*.

398

399 Fig. 3 Behavioral sequence of the male mating behavior in *Panorpa japonica*.

400

401 Fig. 4 Mating duration of the three mating tactics. The error bars on the graphs
402 represent the standard error.

403

404 Fig. 5 Comparisons of FA and body size between the two mating tactics (by mixed
405 ANOVA). The top graph shows the comparisons of the absolute value of FA between
406 the two mating tactics, and the bottom graph shows the comparisons of body size
407 between the two mating tactics.

408

409 Fig.6 Comparisons of male grasper size (top graph) and grasper base size (bottom
410 graph) for the nuptial gift (A) and feeding mating (B) tactics.

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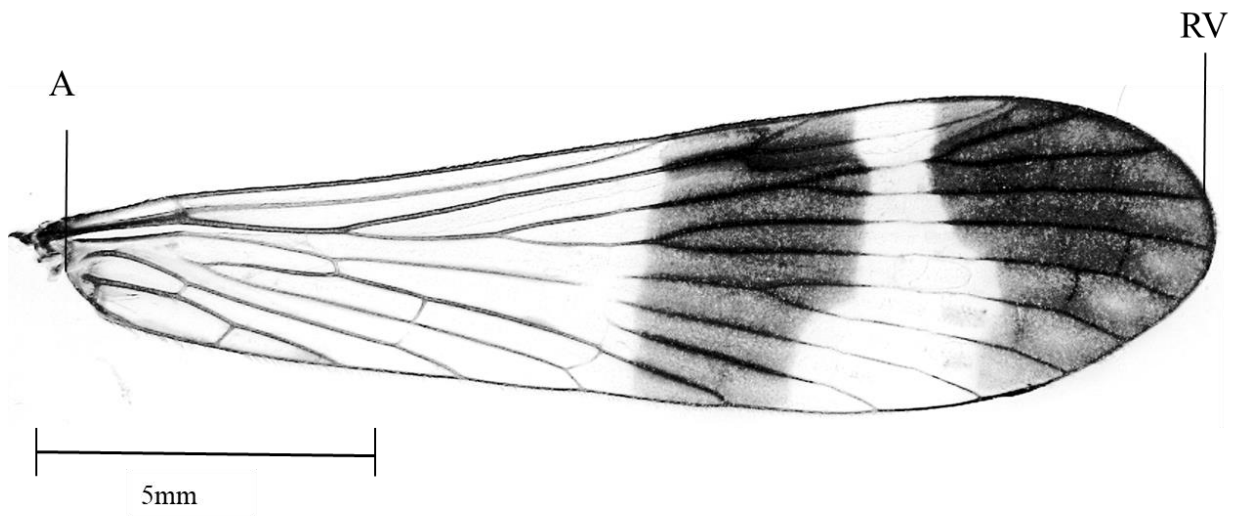


Fig.1

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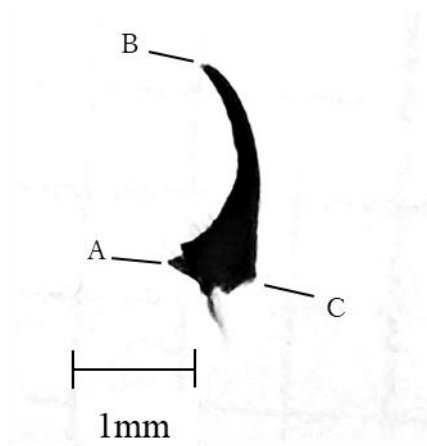


Fig.2

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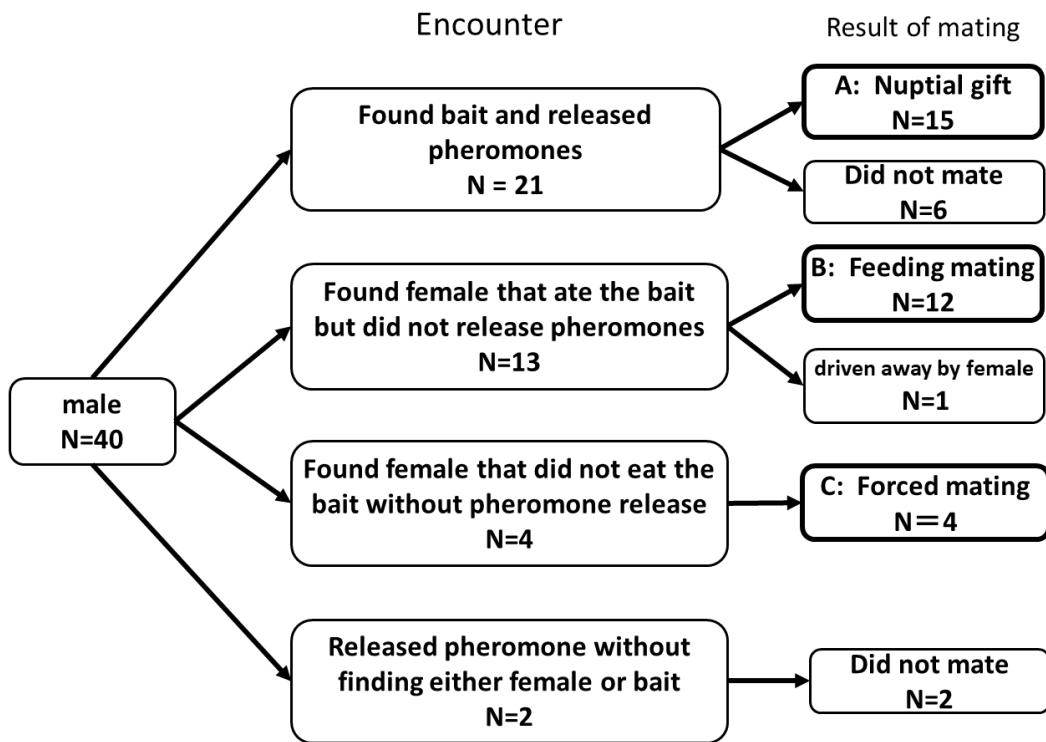


Fig.3

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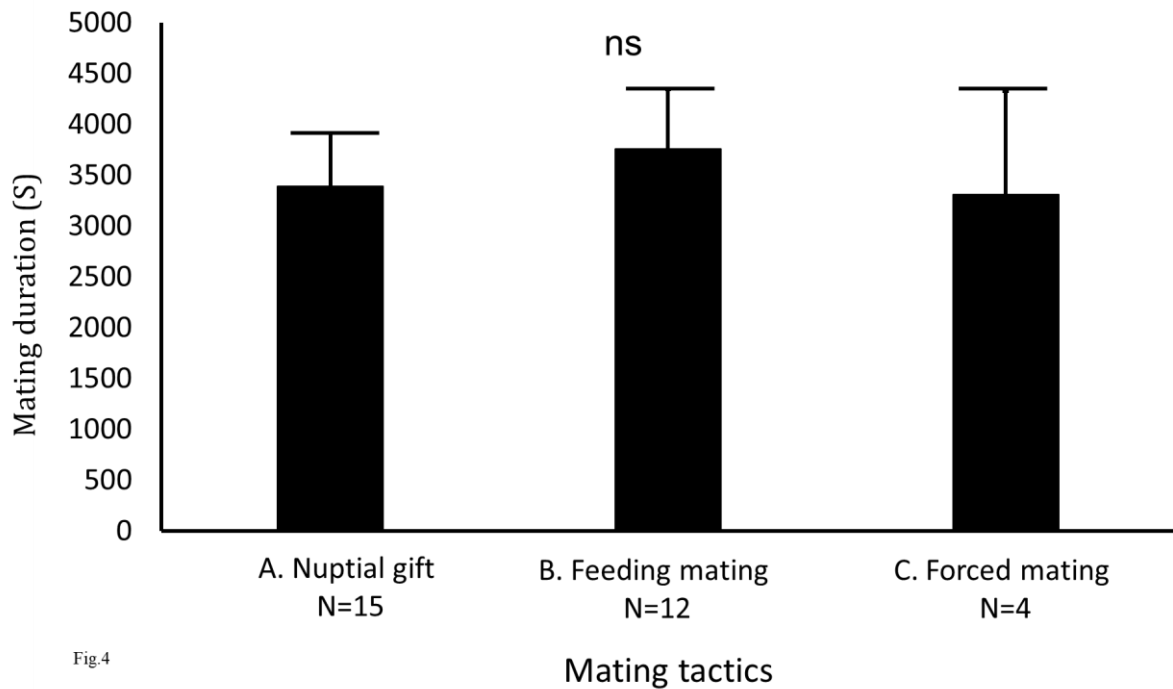


Fig.4

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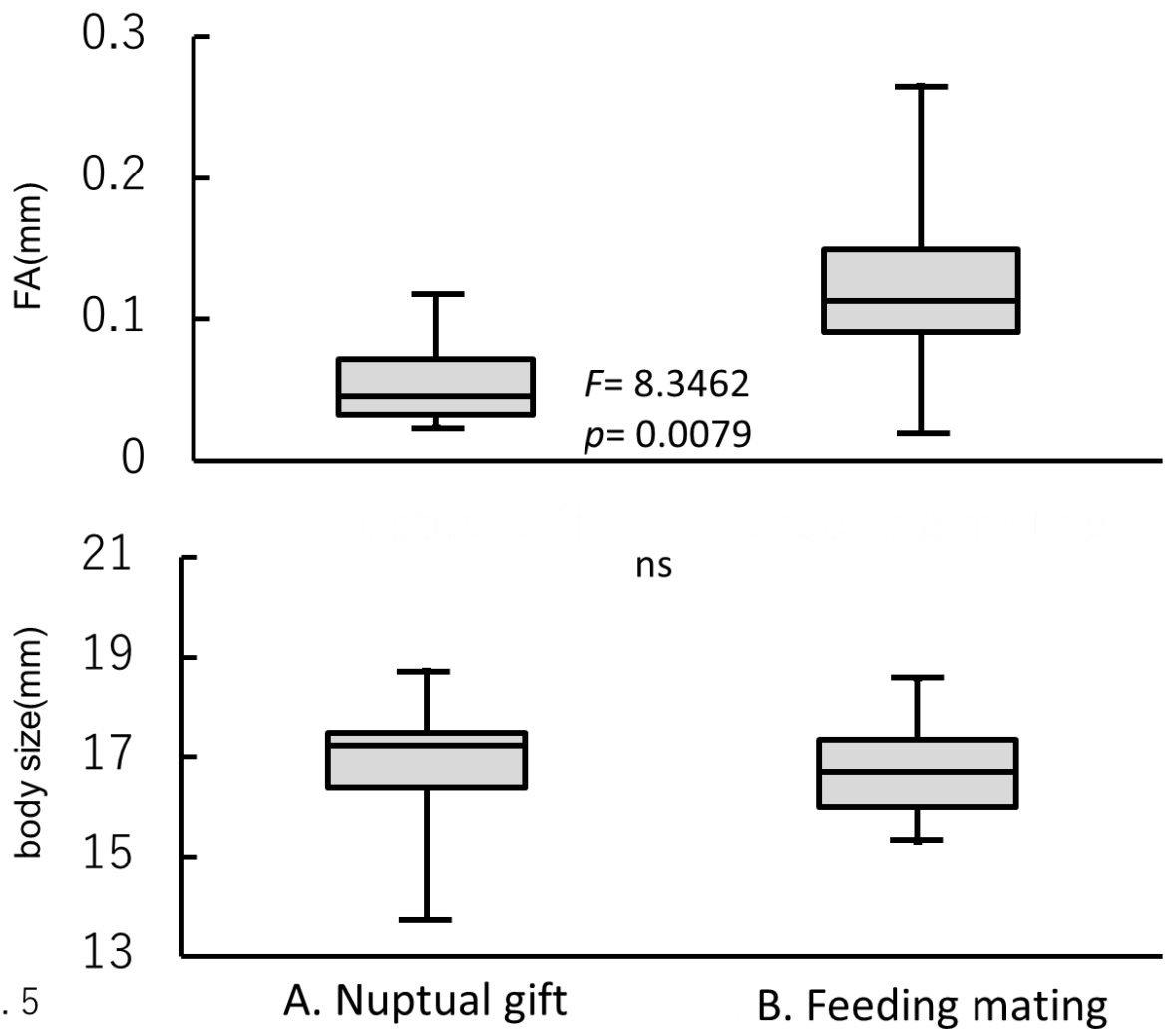


Fig. 5

439

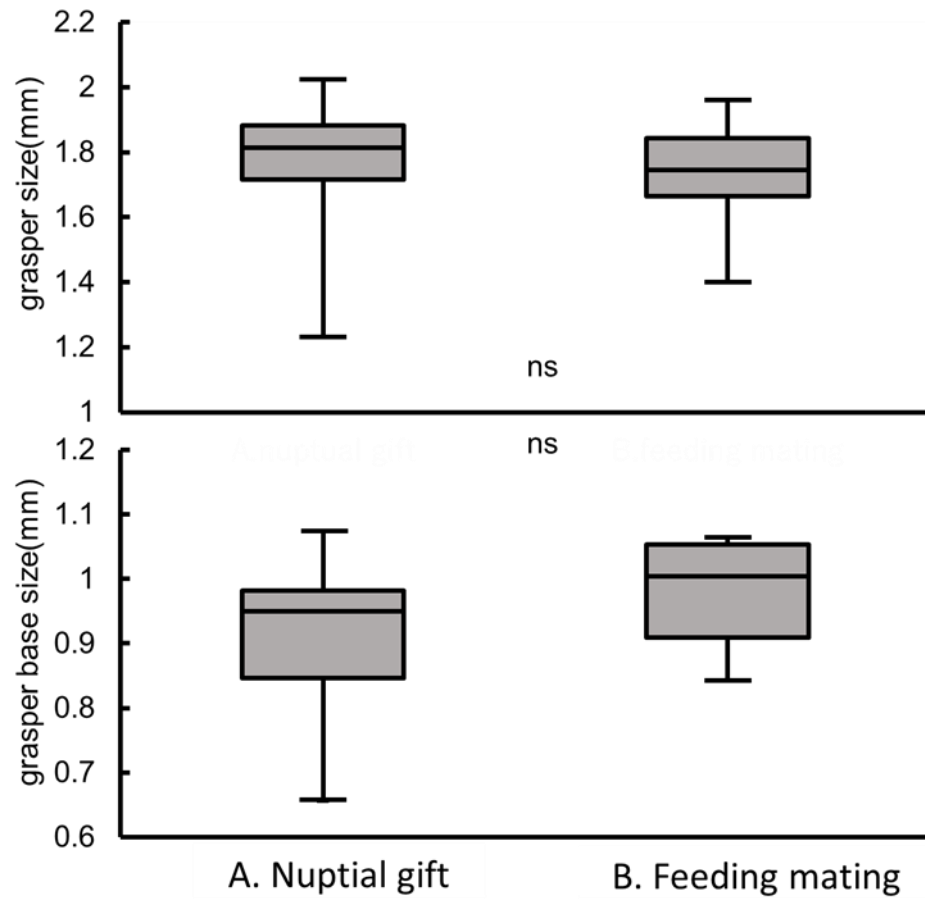


Fig.6

440