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1 **Asymmetry of mating behaviour affects copulation success in two stored**  
2 **product beetles**

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23 **Key message**

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- 25 • There is no information on lateralization (i.e., left–right asymmetries in the  
26 brain and behaviour) in Coleoptera.
- 27 • We investigated lateralization of mating traits in two pests of stored products,  
28 *Sitophilus oryzae* and *Tribolium confusum*.
- 29 • Male beetles exhibited population-level left-biased copulation approaches of  
30 potential mates.
- 31 • Males performing left-biased copulation attempts achieved higher mating  
32 success over right-biased males.
- 33 • The quantification of mating displays could allow comparisons with other  
34 strains, allowing the evaluation of the impact of rearing on mating traits.

35

36 **Author Contribution Statement:** GB conceived and designed research. DR  
37 conducted experiments. All authors contributed new reagents and/or analytical tools.  
38 GB, DR, CS, NKG, CGA and AC analysed data. GB, NGK, CGA and AC wrote the  
39 manuscript. All authors read and approved the manuscript.

40

41

42 **Abstract**

43

44 Lateralization (i.e., left–right asymmetries in the brain and behaviour) has  
45 been documented in all vertebrate classes, while evidences for invertebrates are  
46 limited. To the best of our knowledge, there is no information about behavioural  
47 asymmetries in the Order Coleoptera. In this research, we investigated lateralization  
48 of mating traits in two major pests of stored products, the rice weevil, *Sitophilus*  
49 *oryzae* (Coleoptera: Curculionidae) and the confused flour beetle, *Tribolium*  
50 *confusum* (Coleoptera: Tenebrionidae). Both *T. confusum* and *S. oryzae* males  
51 showed population-level left-biased copulation approaches of potential mates.  
52 Interestingly, *T. confusum* and *S. oryzae* males performing left-biased copulation  
53 attempts achieved higher mating success over right-biased males. Furthermore, *S.*  
54 *oryzae* males periodically exhibited s typical head wagging behaviour, which  
55 consists of waving the rostrum laterally across the female thorax and then resting the  
56 rostrum on the mid thorax. This behaviour was right-biased, even in the majority of  
57 males, which previously showed left-biased copulation attempts. The quantification  
58 of mating displays could allow comparisons with other strains, in order to evaluate  
59 the impact of a given host or of the rearing methods on the mating traits of *S. oryzae*  
60 and *T. confusum*. Overall, this is the first report of lateralization of mating traits in  
61 Coleoptera. Furthermore, this research adds basic knowledge to the reproductive  
62 behaviour of *S. oryzae* and *T. confusum*.

63

64 **Keywords:** behavioural asymmetries; courtship; foodstuff beetles; mass rearing;  
65 *Sitophilus oryzae*; *Tribolium confusum*

66

67       **1. Introduction**

68

69           Lateralization (i.e., left–right asymmetries in the brain and behaviour) has  
70 been documented in all vertebrate classes (Bisazza et al. 1998a; Vallortigara 2000;  
71 Vallortigara et al. 1999, 2011; Rogers and Andrew 2002; Vallortigara and Rogers  
72 2005; MacNeilage et al. 2009; Rogers et al. 2013a). Left–right asymmetries in the  
73 brain and behaviour may enhance brain efficiency in cognitive tasks involving  
74 concurrently but differently both hemispheres (Rogers et al. 2004).

75           Limited research efforts have been conducted to shed light about this  
76 fascinating issue in invertebrate species (Frasnelli et al. 2012a). In insects, there are  
77 evidences for lateralization in movement in foraging columns by ants (Vallortigara &  
78 Rogers 2005), antennal contacts in ants (Frasnelli et al. 2012b), lateralization of visual  
79 learning in honeybees (Letzkus et al. 2008) and leftward turning bias during nest site  
80 exploration in ants (Hunt et al. 2014). Recent research efforts on behavioural  
81 asymmetries of aggressive traits have been conducted in mosquitoes (Benelli et al.,  
82 2015a), tephritid flies (Benelli et al. 2015b; Benelli et al. 2015c), blowflies (Romano  
83 et al., 2015), and honeybees (Rogers et al. 2013b). However, studies on lateralization  
84 of courtship and mating behaviour are scarce. Concerning behavioural asymmetry of  
85 courtship and mating displays in invertebrates, it has been recently reported that in  
86 the parasitic wasp *Leptomastidea abnormis* (Girault) (Hymenoptera: Encyrtidae), the  
87 males showed a right biased display of antennal tapping during courtship behaviour,  
88 (Romano et al. 2016). As regards to tephritid flies, *Bactrocera oleae* (Rossi) males  
89 show a lateral bias during courtship and mating behaviour, courting females more  
90 frequently from the left than the right, front, or backside (Benelli et al. 2015c).

91 Furthermore, behavioural asymmetries in mating were reported for the earwig  
92 *Labidura riparia* Pallas (Dermaptera: Labiduridae), in which the males having two  
93 penises, hold their intromittent organs in the “right-ready” state when not mating, as  
94 well as when mating (Kamimura 2006).

95 Besides insects, further evidences of lateralization of courtship and mating  
96 behaviour in invertebrates were reported also in some molluscs. For instance, in the  
97 simultaneous reciprocal hermaphrodite *Cornu aspersum* (Müller) (Pulmonata:  
98 Helicidae), it has been showed that the electrical stimulation of the right  
99 mesocerebrum evoked genital eversion, suggesting that neurons of the right  
100 mesocerebrum play a key role in controlling the mating behaviour of this mollusc  
101 (Koene et al. 2000). In addition, Davison et al. (2009) observed lateralization of pre-  
102 copulation behaviour in the pond snail *Lymnaea stagnalis* (L.) (Pulmonata:  
103 Lymnaeidae), where the asymmetry of male circling behaviour preceding mating  
104 corresponds to the sinistral or dextral shell coil, or chirality, of the snail, and is  
105 apparently controlled by a maternal locus. To the best of our knowledge, there is no  
106 information about behavioural asymmetries in the Order of Coleoptera.

107 The confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera:  
108 Tenebrionidae) is a long-live species that can seriously and rapidly infest stored-  
109 products (Pedersen 1992; Verheggen et al. 2007; Mason and McDonough 2012). It  
110 is regarded as a secondary colonizer since it cannot easily develop in sound grain  
111 kernels (Storey 1987; Trematerra et al. 2000). *T. confusum* is considered as one of  
112 the most tolerant stored-products species to several contact insecticides that are used  
113 as grain protectants (Kavallieratos et al. 2011, 2013; Athanassiou and Kavallieratos  
114 2014; Kavallieratos et al. 2015).

115           The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) is an  
116 extremely destructive beetle that infects different types of stored products worldwide,  
117 (Aitken 1975; Hill 2002; Mason and McDonough 2012). As a primary pest, it is  
118 capable of infesting unbroken grain kernels in the adult stage whereas its larvae are  
119 fed and complete their development inside kernels (Trematerra et al. 2000; Mason  
120 and McDonough 2012). Given that the immature development of this species is  
121 completed within kernels, larvae are not vulnerable to contact insecticides that are  
122 applied on the external kernel part (Arthur and Throne 2003). Its adults are long-  
123 lived, the females lay eggs throughout their lifespan (Hill 2002) and it has developed  
124 resistance to several insecticides (Haliscak and Beeman 1983; Benhalima et al.  
125 2014), indicates that the presence of *S. oryzae* requires particular attention in storage  
126 facilities.

127           Both *T. confusum* and *S. oryzae* have been found to coexist and co-infest the  
128 product in many storage facilities, exhibiting an ecological succession in their  
129 infestation patterns (Buchelos and Athanassiou 1993; Athanassiou et al. 2006).  
130 Infestation or contamination of these commodities by the primary colonizer *S. oryzae*  
131 may easily make them vulnerable to infestation by the secondary colonizer *T.*  
132 *confusum* that will increase the infestation further (Trematerra et al. 2000). Thus, the  
133 presence of *T. confusum* in high densities in grain kernels can be used as a reliable  
134 indicator of potentially serious qualitative degradation of stored grains.

135           In both *T. confusum* and *S. oryzae*, the males produce an aggregation  
136 pheromone, and females are highly promiscuous, engaging prolonged mating  
137 interactions with multiple males (Fedina and Lewis 2008; Flay and Wang 2010).  
138 Thus, even if *T. confusum* and *S. oryzae* are considered non-social, we hypothesise

139 that population-level behavioural asymmetries may occur in these two beetle species  
140 since they have frequent and prolonged mating approaches, which include repeated  
141 male-male competition for females, mate choice by females and male-female sexual  
142 interactions (Ghirlanda and Vallortigara 2004; Frasnelli et al. 2012a; Benelli et al.  
143 2015a). At the same time, there are interspecific interactions between these species,  
144 regarding the behavioural responses of each species to the aggregation pheromone of  
145 the other species (Athanassiou et al., 2006). In this research, we carried out an  
146 experiment to test the presence of population-level behavioural asymmetries for  
147 different mating traits, which is correlated with higher male mating success. For both  
148 *T. confusum* and *S. oryzae*, an ethogram depicted the lateralized courtship and mating  
149 behaviour phases quantifying their mating success in relation to the biases in  
150 orientation approaches.

151

## 152 **2. Materials and methods**

153

### 154 2.1. Ethics statement

155

156 This research adheres to the guidelines for the treatment of animals in  
157 behavioural research and teaching (ASAB/ABS 2014). All treatments of  
158 experimental animals complied with the laws of the country (Italy) in which they  
159 were performed (D.M. 116192), as well as European Union regulations (European  
160 Commission, 2007). No permits were required by the Italian government for  
161 experiments involving stored product beetles. All experiments were based on  
162 behavioural observation. Beetles were treated as gently as possible given the

163 constraints of the experimental design. None were injured or killed during  
164 the experiments. The health of every insect was constantly assessed by checking  
165 that it fed and behaved normally.

166

## 167 2.2. Beetle rearing

168

169 *T. confusum* was reared on wheat flour including 5% brewer's yeast (by weight)  
170 at 27 °C and 60% RH in continuous darkness. The cultures, initially collected from  
171 Greek storage facilities, have been kept for more than 10 years, initially at Benaki  
172 Phytopathological Institute and later at the Agricultural University of Athens.  
173 *Tribolium confusum* pupae of the same age were separately placed in 30 ml plastic cups,  
174 sexed according to Park (1934) and kept at the same conditions until adult emergence.  
175 The emerged *T. confusum* individuals were kept separately in the same cups under the  
176 same conditions and fed with wheat flour kernels till the beginning of the experiments.  
177 The closure of the cups had a hole covered with gauze for adequate aeration.

178 A wild strain of *S. oryzae*, originally collected in 2015 from a horizontal-type  
179 (flat) storeroom in Attica (Greece) filled with hard wheat, *Triticum durum* Desf., was  
180 reared on whole hard wheat kernels at 27 °C and 60% relative humidity (RH) and in  
181 continuous darkness at the Agricultural University of Athens. Given that *S. oryzae* is an  
182 internal feeder, adults of mixed sex and age were left to infest whole-wheat kernels for a  
183 period of 2 weeks before the beginning of the experiment. Considering the life cycle of  
184 *S. oryzae* (Hill 2002), the 2-weeks period of insect infestation was adequate to obtain  
185 kernels with larvae. After the 2-weeks period, the insects were removed from the culture  
186 and kernels kept separately in 30 ml plastic cups. After emergence, males and females



187 were sexed according to the shape of the rostrum (Halstead 1963), kept separately in the  
188 same cups, under the same conditions, and fed with wheat kernels till the beginning of  
189 the experiments. The closure of the cups had a hole covered with gauze for adequate  
190 aeration.

191

### 192 2.3. Behavioural asymmetries during mating interactions

193

194 Experiments were conducted from December 2015 to January 2016 using virgin  
195 mature males and females of *T. confusum* and *S. oryzae*. *T. confusum* can start lay fertile  
196 eggs 114-20 h after eclosion while it can be fertilized 17-20 h after eclosion (Dawson  
197 1964). *Sitophilus oryzae* needs 4 days to reach sexual maturity (Holloway and Smith  
198 1987).

199 All observations were carried out in a Petri dish arena (diameter 50 mm; height:  
200 10 mm) from 11.00 to 19.00 h, at 27°C and 60% RH. After recognizing the sex of the  
201 beetles the individuals prepared for testing were exposed for three hours to natural light  
202 conditions in Petri dishes (diameter 50 mm) and tested under natural photoperiod. The  
203 experimental arena was surrounded by a white wall of filter paper (Whatman n.1, height  
204 30 cm) in order to avoid that visual cues from the observer impacted the behaviour of  
205 tested beetles (Romano et al. 2016).

206 Both for *T. confusum* and *S. oryzae*, the mating behaviour sequence was  
207 studied transferring a virgin male and a virgin female into the testing arena and  
208 visually tracking them by an observer for 60 min, or until the end of the sexual  
209 interaction, if any. For each mating pair, the direction preference of males  
210 performing their first copulation attempt towards females was observed (*sensu*

211 Benelli et al. 2015c). In addition, we noted the duration of the following phases: (i)  
212 mate recognition (i.e., time spent by the male chasing and approaching the female),  
213 (ii) precopula (i.e., time spent by the male performing mounting attempts on the  
214 female, until genital contact) and (iii) copula (i.e., from the male's insertion of the  
215 aedeagus into the female genital chamber until genital disengagement), as well as (iv)  
216 the duration of the whole courtship and mating sequence. We also observed extended  
217 copulation occurring when the male was accepted by the female and the aedeagus  
218 penetration follows for at least 60 s, to be sure that aedeagus effectively remains in  
219 the female without rejection enhancing male mating success (Benelli et al. 2014).  
220 The *S. oryzae* male approaching a female periodically exhibits characteristic head  
221 wagging behaviour, which consists of waving the rostrum laterally across the female  
222 thorax and then resting the rostrum on the mid thorax (Holloway and Smith 1987).  
223 Therefore, in our experiments we also observed which side of the female's body was  
224 preferred by the male for rostral rubbing.

225 Overall, 129 mating pairs of *T. confusum* and 135 mating pairs of *S. oryzae* were  
226 observed. For both beetle species, males and females that did not engage in any  
227 courtship approach or stayed motionless for more than 60 min were discarded. For  
228 laterality observations, only females that were free in the middle of the arena when  
229 they were approached by males were evaluated (Romano et al. 2016), because  
230 females located close to the sides of the arena would affect male directional  
231 approaches. Since the presence of food is not necessary for mating in *T. confusum*  
232 and *S. oryzae* as it has been shown by previous studies (Wojcik 1969; Boles 1974),  
233 no kernels or other food sources were provided in the arena in order to avoid any

234 constrain affecting the orientation approach of males. One hundred fifteen mating  
235 pairs of *T. confusum* and *S. oryzae* were considered for behavioural analysis.

236

#### 237 2.4. Data analysis

238

239 For each tested species, laterality differences between the numbers of beetles  
240 using left- or right-biased copulation attempt, as well as left- or right-biased head  
241 wagging, during the courtship and mating behaviour were analysed by JMP 7 (SAS  
242 1999) using a weighted generalized linear model with binomial distribution:  $y = X\beta$   
243  $+ \epsilon$  where  $y$  is the vector of the observations (i.e., successful or not successful  
244 mating),  $X$  is the incidence matrix,  $\beta$  is the vector of fixed effect (i.e., direction of the  
245 copulation attempt or head wagging) and  $\epsilon$  is the vector of the random residual  
246 effect. A probability level of  $P < 0.05$  was used for the significance of differences  
247 between values.

248 Data concerning the duration of mate recognition, precopula and copula, as  
249 well as the duration of the whole mating sequence were analysed using a general  
250 linear model with one factor (i.e., direction of the copulation attempt or head  
251 wagging) (JMP 7, SAS 1999). A probability level of  $P < 0.05$  was used for the  
252 significance of differences between values. Data normality was checked using  
253 Shapiro–Wilk test ( $P < 0.05$ ). The variance between values was analysed with  
254 Fisher’s F-test ( $P < 0.05$ ).

255

## 256 **Results**

257

258 The results of the present study clearly indicate that both *T. confusum* and *S.*  
259 *oryzae* males showed left-biased copulation in their orientation to potential mates  
260 (**Figs. 1 and 2**). However, these behavioural trends are highly moderated by several  
261 factors, which have a dissimilar effect for the two species tested.

262 In *T. confusum*, the mean duration of mate recognition ( $F_{2,112}=0.197$ ;  
263  $P=0.821$ ), precopula ( $F_{2,112}=0.299$ ;  $P=0.742$ ), and copula ( $F_{2,112}=0.882$ ;  $P=0.417$ ) did  
264 not differ between males approaching females from the left, right or backside, while  
265 significant differences were detected for the duration of whole mating sequence of  
266 males approaching females from the left, right or backside ( $F_{2,112}=15.336$ ;  $P<0.001$ )  
267 (**Fig. 3**).

268 In *S. oryzae*, no differences were found in the mean duration of mate  
269 recognition ( $F_{3,111}=0.983$ ;  $P=0.403$ ) and precopula ( $F_{3,111}=1.668$ ;  $P=0.178$ ) of males  
270 approaching females from the left, right or backside, while significant differences  
271 were detected for the duration of copula ( $F_{3,111}=8.783$ ;  $P<0.001$ ) and whole mating  
272 sequence of males approaching females from the left, right, frontal backside  
273 ( $F_{3,111}=6.290$ ;  $P<0.001$ ) (**Fig. 4**).

274 Interestingly, both in *T. confusum* and *S. oryzae*, males performing left-biased  
275 copulation attempts included many that were in copula longer than 60 s, over right-  
276 biased males ( $\chi^2_1=34.272$ ;  $P<0.001$ ;  $\chi^2_1=10.972$ ;  $P<0.001$ , respectively) (**Figs. 5 and**  
277 **6**). In addition, *S. oryzae* males periodically exhibited a typical head wagging  
278 behaviour, which consists of waving the rostrum laterally across the female thorax  
279 and then resting the rostrum on the mid thorax (Holloway and Smith 1987). This  
280 behaviour was right-biased (**Fig. 7**), even in the majority of males which previously  
281 showed left-biased copulation attempts ( $\chi^2_2=13.361$ ;  $P<0.001$ ) (**Fig. 8**).

282 Furthermore, the mean duration of mate recognition ( $F_{2,112}=0.339$ ;  $P=0.713$ ),  
283 precopula ( $F_{2,112}=0.726$ ;  $P=0.486$ ), copula ( $F_{2,112}=1.890$ ;  $P=0.153$ ), and whole  
284 mating sequence ( $F_{2,112}=2.725$ ;  $P=0.079$ ) did not differ between males performing  
285 left side, right side or no head wagging (**Fig. 9**).

286

## 287 **Discussion**

288

289 To our knowledge, this is the first study that investigated the presence of  
290 behavioural asymmetries for insects of the Order of Coleoptera. Based on our  
291 findings, stored product beetles, at least in the case of the species tested here, exhibit  
292 lateralized mating patterns. It is generally considered that social species are more  
293 likely to evolve lateralization at the population-level, while solitary species show  
294 more frequently lateralization at an individual level (Ghirlanda and Vallortigara  
295 2004; Vallortigara and Rogers 2005; Ghirlanda et al. 2009; Frasnelli, 2013; Rogers et  
296 al. 2013a). Behavioural asymmetries in a population could be due to the need of  
297 individual asymmetrical organisms to coordinate their behaviour with that of other  
298 asymmetrical organisms (Ghirlanda and Vallortigara 2004). However, population-  
299 level lateralization has been observed in a number of solitary species of invertebrates  
300 (e.g., parasitic wasps; tephritids, drosophilids, water bugs, spiders, crabs, snails,  
301 cuttlefish and squids) and it has been hypothesized that interactions such as mating,  
302 fighting and/or escape responses are involved in the behavioural asymmetries found  
303 in these species (Frasnelli et al. 2012a; Romano et al. 2016). Concerning behavioural  
304 asymmetry of courtship and mating displays in invertebrates, few recent examples  
305 focused on several invertebrate species, including gastropods (Davinson et al. 2009;

306 Koene et al. 2000), earwigs (Kamimura 2006), olive fruit flies (Benelli et al. 2015c)  
307 and parasitic wasps (Romano et al. 2016).

308         To our perception the scenario hypothesised by Frasnelli et al. (2012a) about  
309 the role of interactions such as mating, fighting and/or escape responses, and the way  
310 that these key characteristics are involved in the consolidation of behavioural  
311 asymmetries in arthropods may apply also to *S. oryzae* and *T. confusum*. Indeed, the  
312 laterality biases observed when males performed copulation attempts and head  
313 wagging behaviour may be connected to the prolonged interactions occurring during  
314 courtship and mating (Benelli et al. 2015a; Romano et al. 2016). Notably, courtship  
315 and mating are social behaviours and, even if *S. oryzae* and *T. confusum* are  
316 considered non-social, mating approaches are frequent and prolonged in these two  
317 species (lasting more than an hour in *S. oryzae*). Furthermore, the lateralized head  
318 wagging behaviour displayed by *S. oryzae* may be linked to the higher production of  
319 olfactory and tactile cues, as well as to the higher presence of sensory structures on  
320 the right side of the female head, as recently highlighted for honeybees (Anfora et al.  
321 2010; Frasnelli et al. 2012a). Nevertheless, it is generally established that, for stored  
322 product beetles, adaptability patterns of virgin adults is directly related with parental  
323 and progeny fitness (Giga and Smith 1995; Trematerra et al. 2013). Hence, in this  
324 context, mating behaviour in stored product beetles is linked with progeny  
325 production and, as a result, infestation patterns.

326         Overall, to the best of our knowledge, this is the first report of lateralization  
327 of mating traits for the Coleoptera Order. Furthermore, this research adds basic  
328 knowledge to the courtship and mating behaviour of *S. oryzae* and *T. confusum*, and,  
329 eventually, in their success in commodity colonization patterns. The quantification of

330 mating displays allows comparisons with other strains, in order to evaluate the  
331 impact of a given host or of the rearing methods on the mating success of *S. oryzae*  
332 and *T. confusum*.

333

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335

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347

### 348 **Conflict of interest**

349

350 The Authors declare no competing interests.

351

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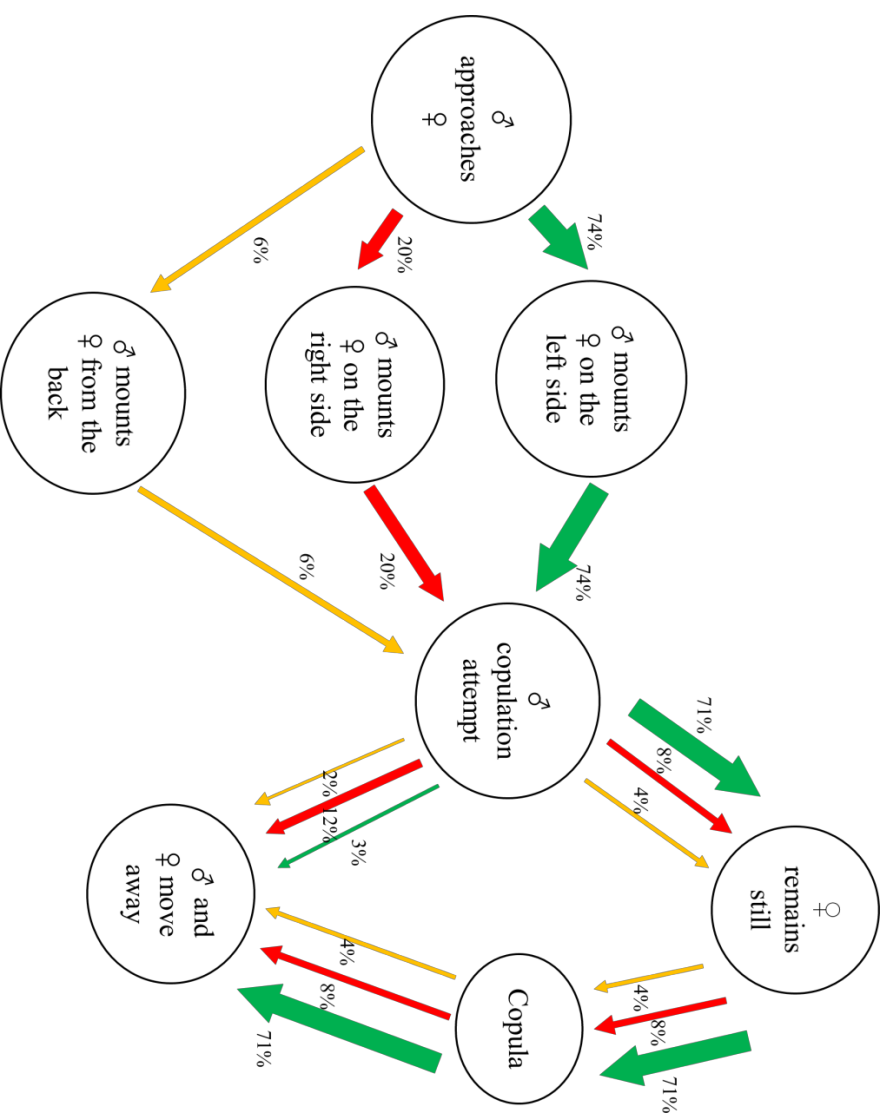
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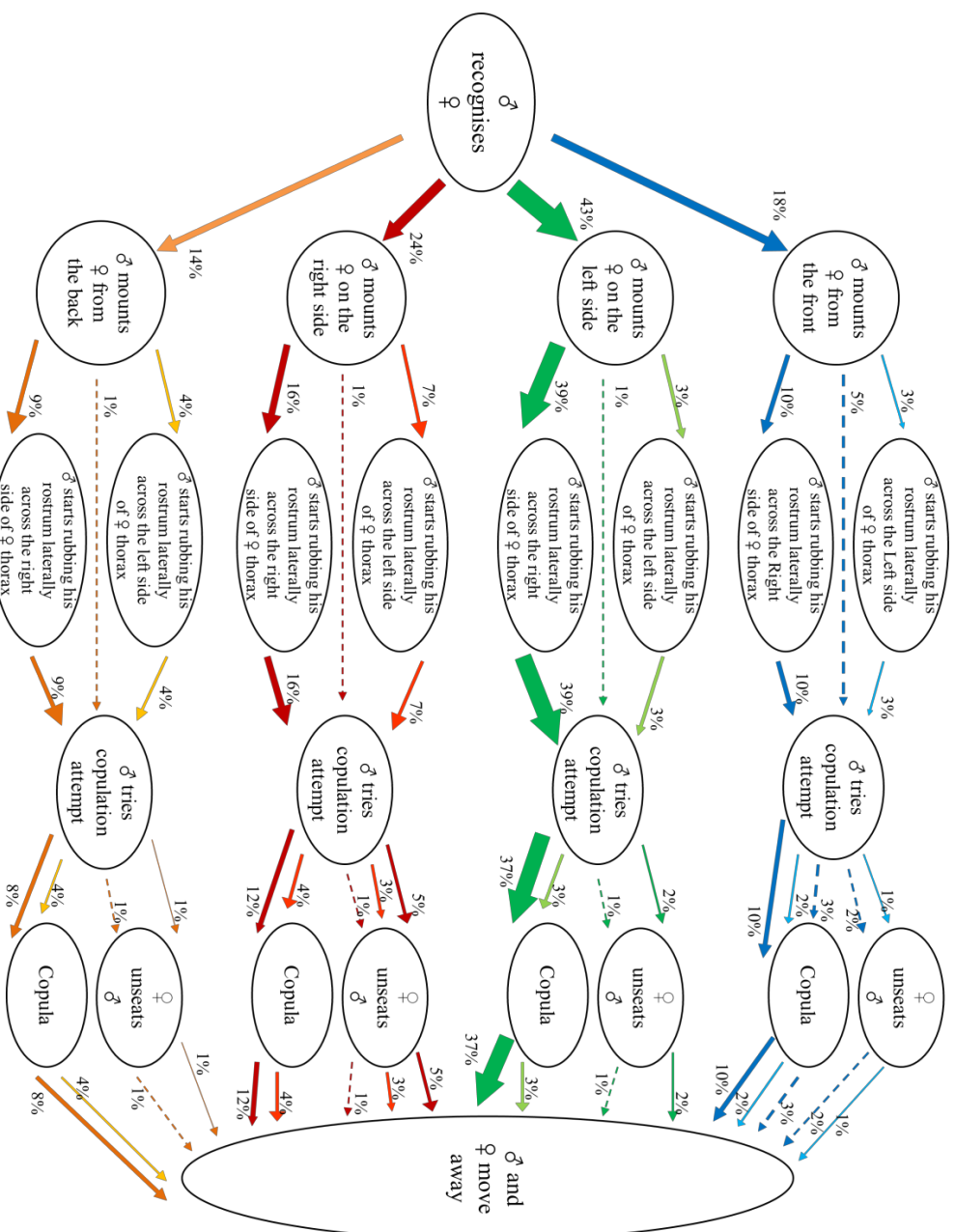
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**Figure 1.** Flow chart of the courtship and mating behaviour of *Tribolium confusum*. The thickness of an arrow indicates the proportion of individuals displaying different behavioural phases. Males mounting females on the left side are represented by green arrows, while the ones mounting on the right side are depicted using red arrows; males approaching from the female backside are shown by yellow arrows.

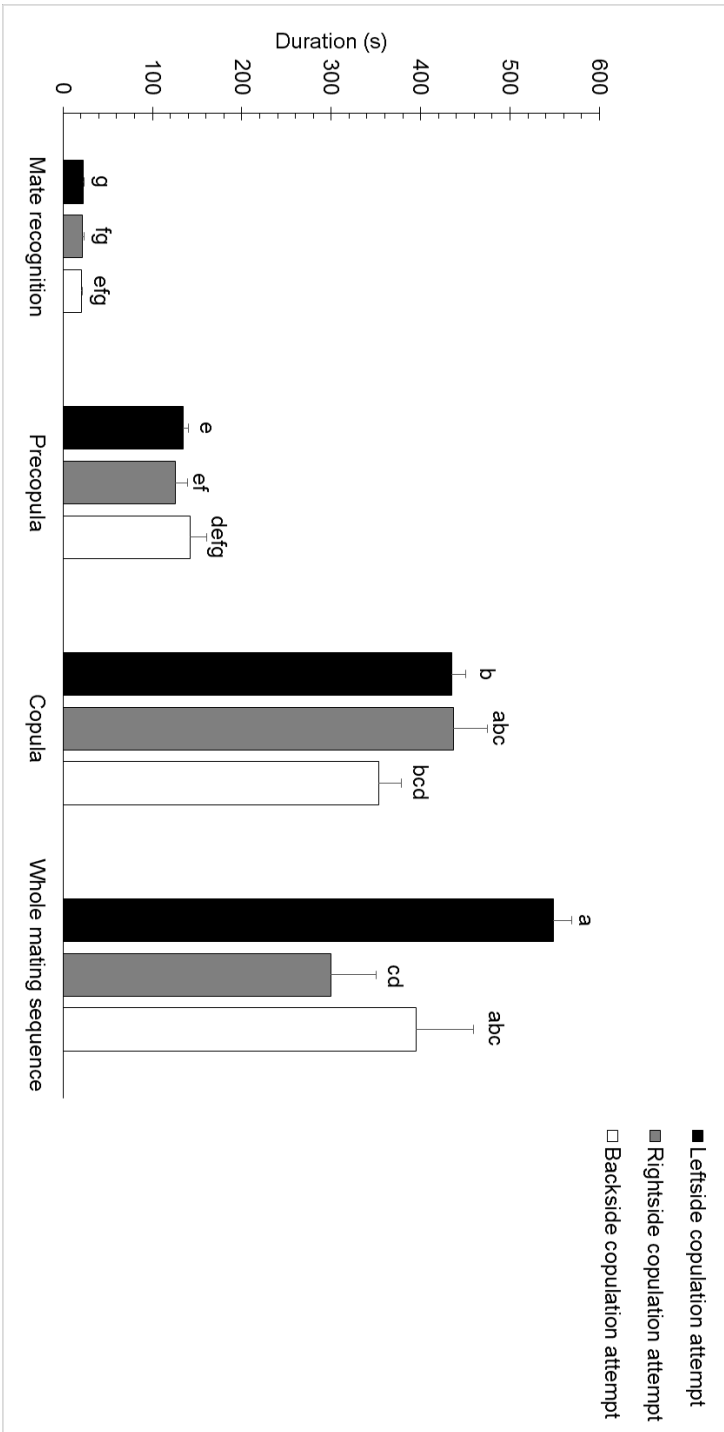


**Figure 2.** Flow chart of the courtship and mating behaviour of *Stophilus oryzae*. The thickness of an arrow indicates the proportion of individuals displaying different behavioural phases. The flow chart shows males mounting females from the front (blue arrows), on the left side (green arrows), on the right side (red arrows) and from the backside (orange arrows). Blue, green, red and orange arrows are then presented as dark- or light-coloured for males starting rubbing their rostrum laterally across the right or left side of female thorax, respectively.

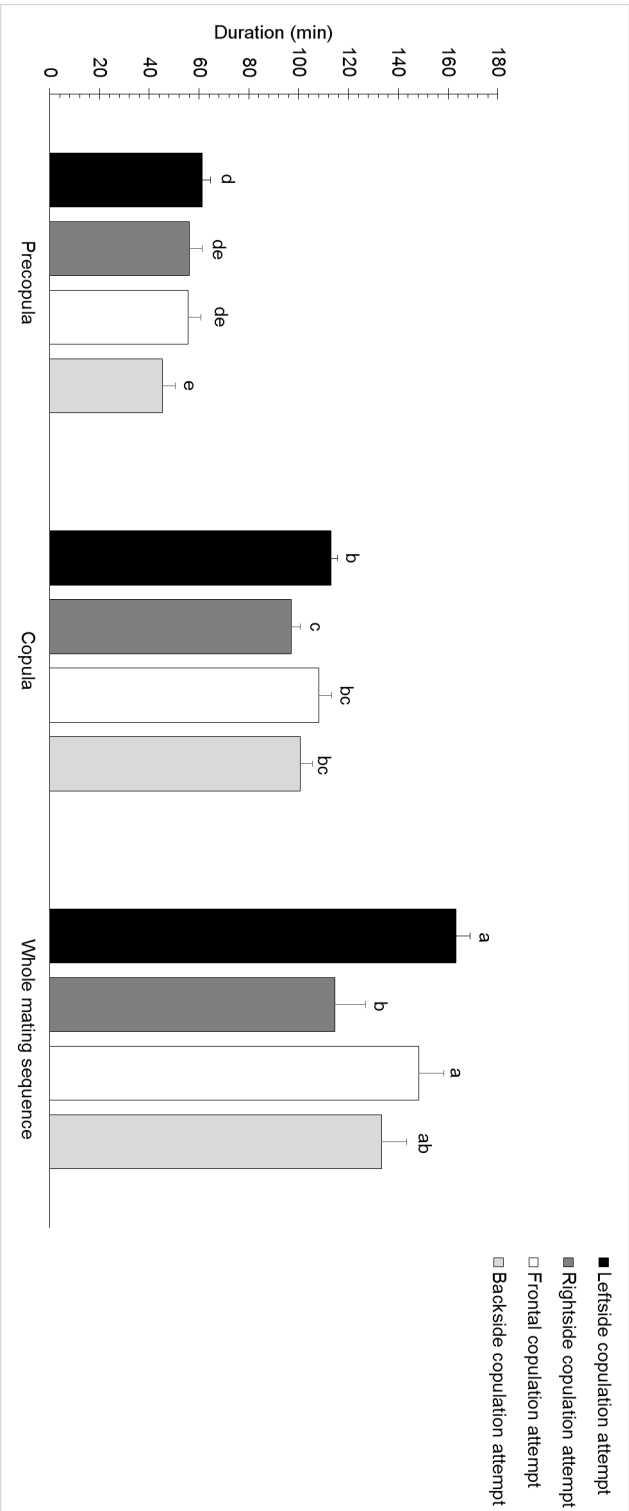




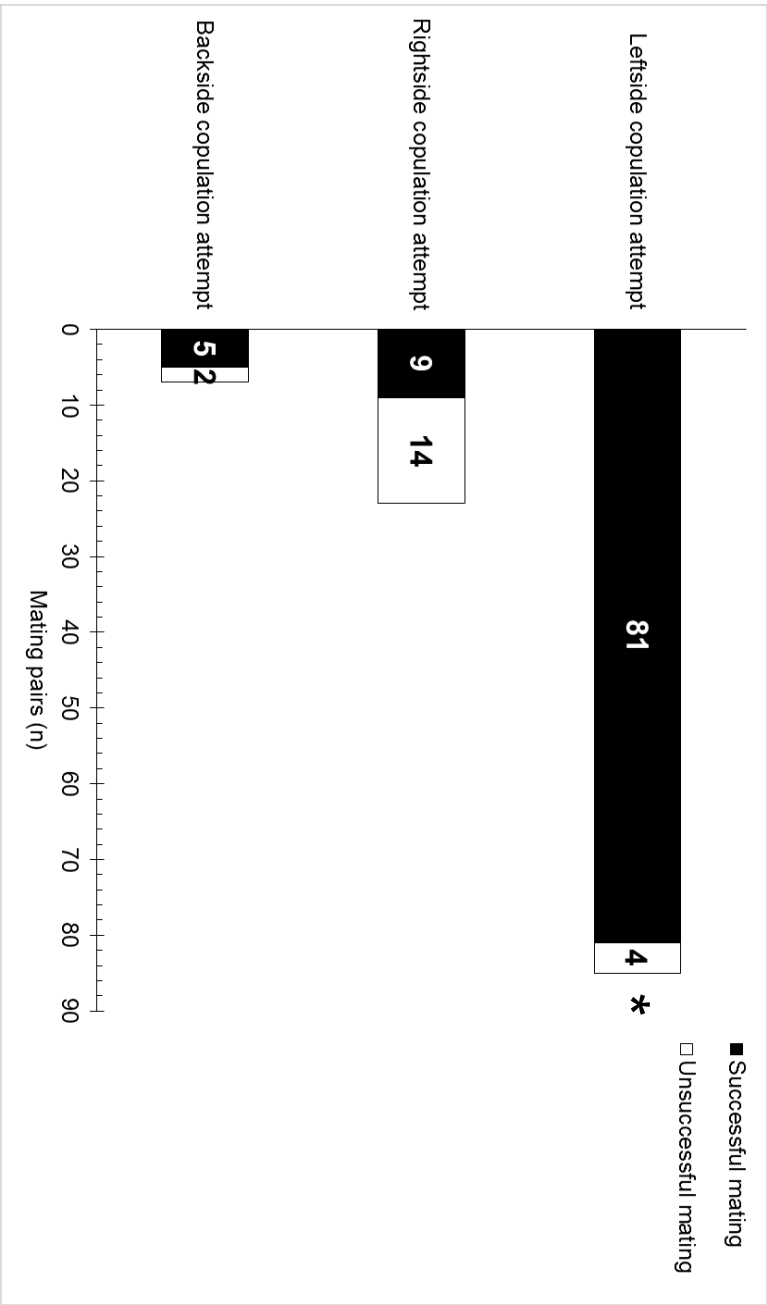
**Figure 3.** Duration of different mating phases in *Tribolium confusum* males performing or not lateralised copulation attempts during the mating approach. T-bars represent standard errors, different letters above each column indicate significant differences (general linear model, normal distribution,  $P < 0.05$ ).



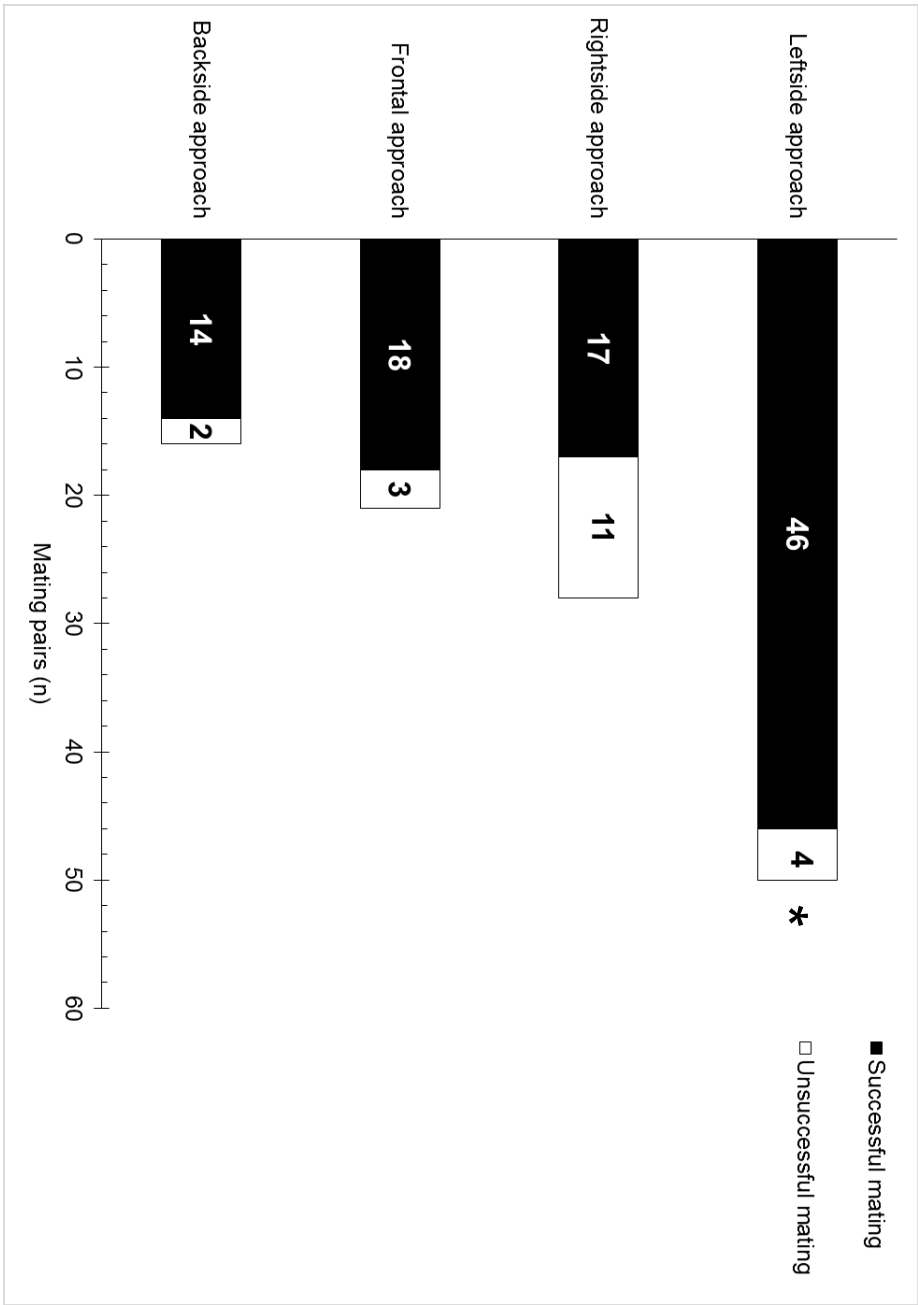
**Figure 4.** Duration of different mating phases in *Stipophilus oryzae* males performing or not lateralised copulation attempts during the mating approach. T-bars represent standard errors; different letters above each column indicate significant differences (general linear model, normal distribution,  $P < 0.05$ ).



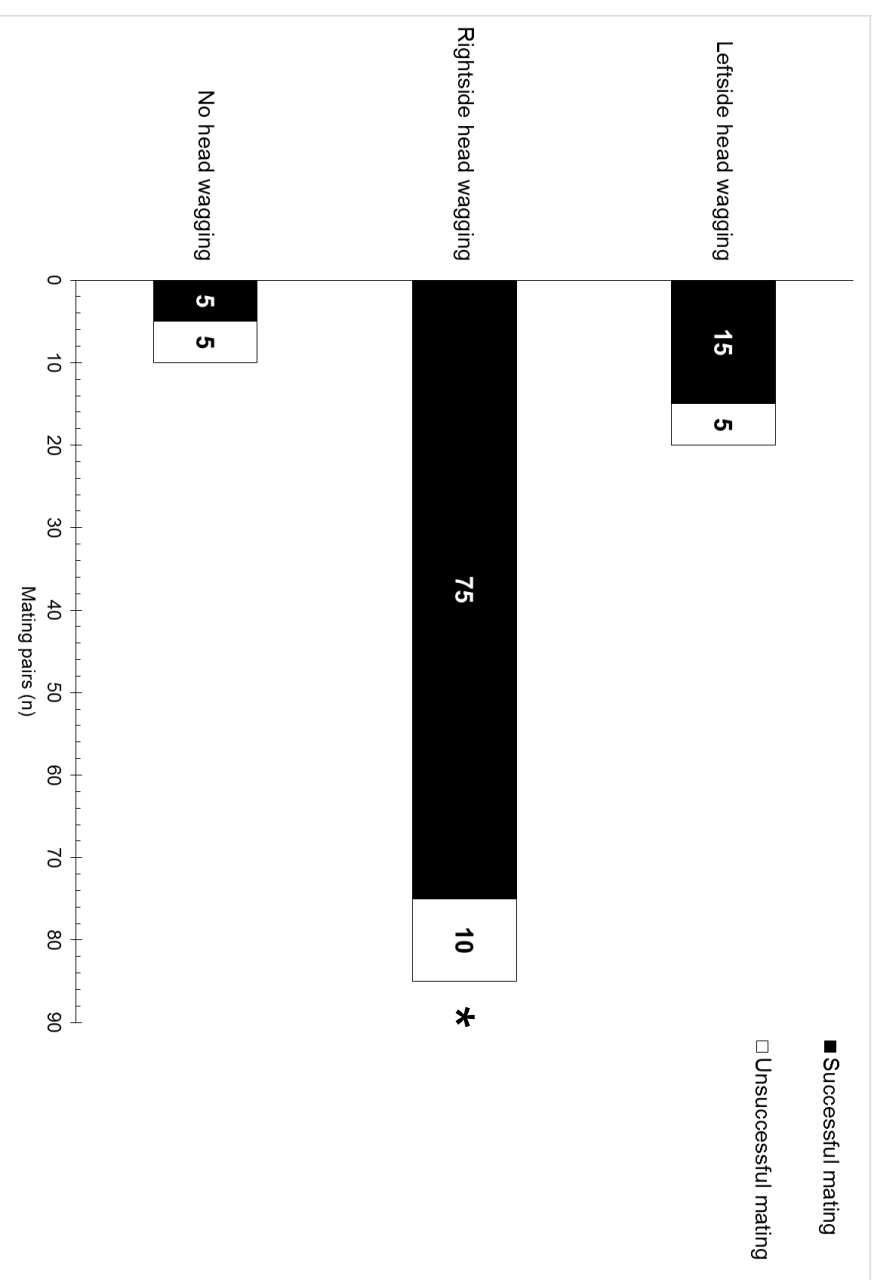
**Figure 5.** Mating success in *Tribolium confusum* males performing or not lateralised copulation attempts during the mating approach. Mating success in *T. confusum* males occurs when the female accepted the male and the aedeagus penetration follows for at least 60 s. The asterisk indicates significant differences among side-biased behaviours (general linear model, binomial distribution,  $P < 0.05$ ).



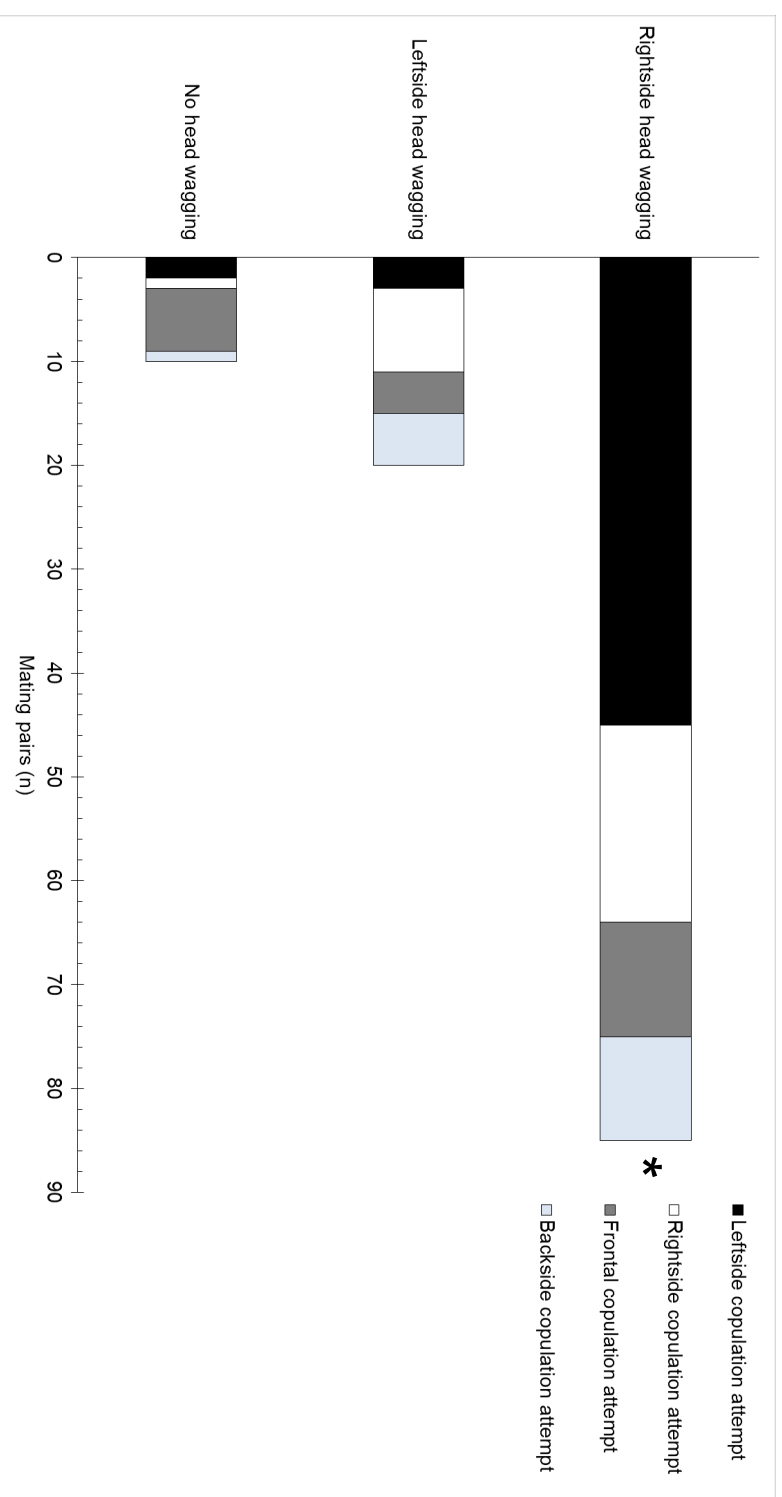
**Figure 6.** Mating success in *Stiphilus oryzae* males performing or not lateralised copulation attempts during the mating approach. The asterisk indicates significant differences among side-biased behaviours (general linear model, binomial distribution,  $P < 0.05$ ).



**Figure 7.** Mating success in *Strophilus oryzae* males performing or not lateralised head wagging behaviour during female mating approaches. The asterisk indicates significant differences among side-biased behaviours (general linear model, binomial distribution,  $P < 0.05$ ).



**Figure 8.** Occurrence of left- and right-biased head wagging movements in *Stiphophilus oryzae* males displaying lateralised copulation attempts during the mating approach. The asterisk indicates significant differences among side-biased behaviours (general linear model, binomial distribution,  $P < 0.05$ ).



**Figure 9.** Duration of different mating phases in *Strophilus oryzae* males performing or not lateralised head wagging behaviour during the mating approach. T-bars represent standard errors; different letters above each column indicate significant differences (general linear model, normal distribution,  $P < 0.05$ ).

