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1 **Multiple behavioural asymmetries impact male mating success in the khapra**
2 **beetle, *Trogoderma granarium***

3

4 Giovanni Benelli ^{a *}, Donato Romano ^b, Nickolas Kavallieratos ^c, Giuseppe Conte ^a,
5 Cesare Stefanini ^d, Marcello Mele ^a, Christos Athanassiou ^e, Angelo Canale ^a

6

7 ^a University of Pisa, Department of Agriculture, Food and Environment, via del
8 Borghetto 80, 56124 Pisa, Italy

9 ^b Sant'Anna School of Advanced Studies, The BioRobotics Institute, viale Rinaldo
10 Piaggio 34, Pontedera, 56025 Pisa, Italy

11 ^c Agricultural University of Athens, Department of Crop Science, Laboratory of
12 Agricultural Zoology and Entomology, 75 Iera Odos str., 11855, Athens, Attica,
13 Greece

14 ^d Department of Biomedical Engineering, Khalifa University, PO Box 127788, Abu
15 Dhabi, UAE

16 ^e University of Thessaly, Department of Agriculture Crop Production and Rural
17 Environment, Laboratory of Entomology and Agricultural Zoology, Phytokou str.,
18 38446, N. Ionia, Magnesia, Greece

19

20 * Corresponding author. University of Pisa, Department of Agriculture, Food and
21 Environment, via del Borghetto 80, 56124 Pisa, Italy. E-mail address:
22 benelli.giovanni@gmail.com (G. Benelli).

23

24 **Key message**

25

- 26 • Little is known on mating behavioural asymmetries in insects, with special
27 reference to beetles
- 28 • We studied mating behavioural asymmetries in the khapra beetle *Trogoderma*
29 *granarium*
- 30 • Four population-level lateralized mating traits were reported in this beetle
- 31 • Male recognition, mounting and female post-copulation kicks were left-biased
- 32 • Left-biased asymmetries in the recognition approach affected male mating
33 duration and success

34

35 **Author contribution statement**

36

37 GB conceived and designed research. All authors conducted experiments and
38 contributed new reagents and analytical tools. GB and GC analyzed data. GB, NK
39 and CA wrote the manuscript.

40

41

42 **Abstract**

43

44 Recently, extensive research has been conducted to shed light on mating
45 behavioural asymmetries in various species of vertebrates. However, little is
46 available on this key issue for invertebrates, especially for species of the order
47 Coleoptera. In the present study, we highlighted the presence of four population-level
48 lateralized mating traits in the mating behaviour of an alien and invasive pest of
49 stored products, the khapra beetle, *Trogoderma granarium* (Dermestidae). Male
50 recognition and mounting, as well as the female post-copulation kicks were left-
51 biased, while male foreleg tapping acts were right-biased. Notably, left-biased
52 asymmetries in the recognition approach led to significant differences in recognition
53 duration, number of head wagging acts, whole mating duration and, most
54 importantly, male mating success. Overall, results reported here represent a rather
55 unique example of four subsequent mating traits lateralized at population-level,
56 which influence the male mating success. From an applied perspective, the
57 quantification of lateralized mating traits is expected to contribute to a better
58 understanding of key biological traits of this species, that can be used for prediction
59 of its spread and population growth parameters.

60

61 **Keywords:** brain laterality; invasive species; stored-product pest; population-level
62 lateralization

63

64

65 **Introduction**

66

67 It is widely recognized that left and right asymmetries in the brain and
68 behaviour (i.e., lateralization) may enhance brain efficiency in cognitive tasks
69 involving concurrently but differently both hemispheres (Vallortigara 2000;
70 Vallortigara and Rogers 2005; Vallortigara et al. 2010; Rogers et al. 2013a, 2013b).
71 However, while extensive research has been conducted to shed light on mating
72 behavioural asymmetries in vertebrates, very few information is available on this
73 key issue for invertebrates (Frasnelli et al. 2012, Frasnelli 2013), especially insect
74 species (Benelli et al. 2015a,b,c; Romano et al. 2016a). Among the insect species
75 tested, Coleoptera are the less-studied, as the presence of behavioural asymmetries
76 during mating approaches has been documented in only two beetle species, both of
77 economic importance at the post-harvest stages of agricultural commodities
78 (Benelli et al. 2016; Romano et al. 2016b).

79 The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae)
80 is considered as a stored-product insect species of high phytosanitary importance, as
81 it is classified among the 100 most invasive species (Hill 2003, Myers and Hagstrum
82 2012; EPPO 2013, 2016). It has spread from India in numerous countries of Africa,
83 Asia and Europe, and it is a quarantine species for many countries with high cereal
84 production, such as USA, Australia and Canada (Aitken 1975, Myers and Hagstrum
85 2012, Athanassiou et al. 2015, EPPO 2016). Larvae of *T. granarium* can be easily
86 spread through international trade, as they feed on variable different commodities of
87 both plant and animal origin (Burges 2008). Moreover, they have the ability to pass
88 through an unusually long diapause that may exceed 8 years when conditions

89 prevailing are not suitable for larval development (Wilches et al. 2016). Also, this
90 species is able to develop within a wide range of temperatures (21-40 °C), and
91 populations decrease significantly when exposed to a temperature of 20°C or below,
92 while at elevated temperatures it can build easily high population densities, as these
93 conditions are unsuitable for the development of other major stored product insect
94 species (Aitken 1975, Burges 2008). Finally, this species can infest very dry stored-
95 products (i.e., 2% moisture content), which are not suitable for the development of
96 other species (Hadaway 1956, Burges 1959, 1962; Peacock 1993; Hagstrum and
97 Subramanyam 2009; Athanassiou et al. 2016). For all the above reasons, *T.*
98 *granarium* is considered a serious threat for grains and other related commodities in
99 specific zones, while the number of its interceptions in quarantine countries has been
100 gradually increased, indicating that this species is spreading (Myers and Hagstrum
101 2012). Considering the pest's plasticity in temperature requirements, *T. granarium*
102 was able to expand in colder climates as in the case of Western Central Europe
103 (EPPO 2016). Apart from the direct losses of stored products, the pest's status
104 becomes more important since it contaminates the commodities with cast skins and
105 hairs of its larvae that may endanger human health (Morison 1925; Pruthi and Singh
106 1950).

107 Previous studies have shown that mating behaviour in major stored-product
108 beetle species is particular and are linked with specific biotic and abiotic conditions,
109 which can seriously affect the concomitant population growth (Benelli et al. 2016,
110 Romano et al. 2016b). For example, in an earlier study, Benelli et al. (2016), reported
111 that there were specific mating patterns of the rice weevil, *Sitophilus oryzae* (L.)
112 (Coleoptera: Curculionidae) and the confused flour beetle, *Tribolium confusum*

113 Jacquelin du Val (Coleoptera: Tenebrionidae), that determine the success of mating,
114 and, apparently, the concomitant progeny production and population growth.
115 Notably, limited information is available on the courtship and mating ecology of *T.*
116 *granarium*, while most of the studies have been focused on its pheromone-based
117 communication (Adeesan et al. 1969; Levinson and Ilan 1970; Rahalkar et al. 1972;
118 Cross et al. 1976; Levinson et al. 1978; Hammack and Burkholder 1981). This
119 species seem to have different population growth patterns, which are directly linked
120 with larval diapause, since diapausing larvae provide adults that are able to produce
121 more eggs and, as a result, to cause rapid population outbursts (Karnavar 1972). Still,
122 mating of *T. granarium* has not been investigated in detail, and the overall
123 behavioural patterns at the adult stage are rather overlooked, despite the quarantine
124 importance of this species. In addition, current knowledge on the presence,
125 magnitude and potential function of behavioural asymmetries in the order Coleoptera
126 is extremely patchy (Benelli et al. 2016; Romano et al. 2016b). Therefore, in this
127 study, we quantified the courtship and mating behaviour of *T. granarium*, showing
128 the presence of population-level behavioural asymmetries for four different mating
129 traits. Furthermore, we highlighted selected behavioural parameters that are
130 correlated with high male mating success in this key noxious species of stored-
131 products..

132

133 **Materials and methods**

134

135 Ethics statement

136

137 This research adheres to the guidelines for the treatment of animals in
138 behavioural research and teaching (ASAB/ABS 2014). All treatments of
139 experimental animals complied with the laws of the country in which they were
140 performed, as well as European Union regulations (European Commission 2007).

141

142 *T. granarium* rearing

143

144 *T. granarium* was reared on whole-wheat kernels at 30 °C and 65% R.H. in
145 continuous darkness at the Laboratory of Agricultural Zoology and Entomology, the
146 Agricultural University of Athens. The insect colony was started in 2014 from insects
147 collected in Greek storage facilities. *T. granarium* pupae of the same age were
148 separately placed in 30 ml plastic cups and sexed according the characters provided by
149 Partida et al. (1969). After emergence, males and females were kept separately in the
150 cups, under the same conditions. The closure of the cups had a hole covered with gauze
151 for adequate ventilation (Athanassiou et al. 2016).

152

153 Behavioural asymmetries during mating interactions

154

155 Virgin mature males and females of *T. granarium* were used in all experiments,
156 which we conducted from June to August 2016. *T. granarium* can start lay eggs 2-3 d
157 after eclosion while it can mate immediately after eclosion (Lindgren et al. 1955, Hill
158 2002). All observations were carried out in a Petri dish arena (diameter 50 mm; height:
159 10 mm) from 11.00 to 19.00 h, at 30°C and 65% RH. We made slightly rough the floor
160 of the Petri dish arena by using sand paper on the plastic surface for 10 s, in order to

161 improve the insect's grip during walking activity. Before the beginning of the
162 experiments, males and females were exposed for 3 h to laboratory light conditions in
163 Petri dishes. The room was illuminated with fluorescent daylight tubes [16:8 (L:D)
164 photoperiod, lights on at 06:00]. Neon tubes (Philips 30W/33) were used, and the light
165 intensity in close proximity of the testing arena was 1000 lux, estimated over the 300-
166 1100 nm waveband using a LI-1800 spectroradiometer (LI-COR Inc., Lincoln, NE,
167 USA), equipped with a remote cosine receptor (Benelli and Canale 2016). A white wall
168 of filter paper (Whatman n.1, height 30 cm) surrounded the experimental arena in order
169 to avoid visual cues from the observer, which might affect the behaviour of the tested
170 beetles (Romano et al. 2016b).

171 *Trogoderma granarium* mating behaviour sequence was visually tracked by
172 an observer after transferring a virgin male and a virgin female into the testing arena
173 and observed for 60 min, or until the end of the sexual interaction, if any (Benelli et
174 al. 2016). Based on preliminary observations, we noticed that *T. granarium* male,
175 during courtship, starts to explore the female body from a side palpating her body
176 with forelegs and pushing her with head, then the male try to mount the female and
177 attempt copulation with several aedeagus tapping acts on the hind part of the
178 abdomen of the female. Copulation occurs when the male inserts the aedeagus into
179 the female genital chamber and stays still on the substrate near the female during
180 genital contact. After copulation, often females unseated and chased away males by
181 kicking them with hind legs and sometimes, head butting of females towards males
182 followed.

183 For each mating pair, we noticed asymmetries exhibited by males in the
184 exploration of the female body side, the foreleg used to palpate female and the

185 female side preference of males attempting to mount females (Benelli et al. 2015;
186 Romano et al. 2016b). Furthermore, any females' bias during the kicking behaviour
187 towards males was also recorded. In addition, we recorded the duration of the
188 following phases: (i) preliminary recognition of the female (i.e., time spent by the
189 male palpating and pushing the female), (ii) precopulation (i.e., time spent by the
190 male performing mounting attempts on the female and aedeagus tapping acts, until
191 genital contact), (iii) copulation (i.e., from the male's insertion of the aedeagus into
192 the female genital chamber until genital disengagement), as well as (iv) the duration
193 of the whole courtship and mating sequence. The number of acts regarding males
194 head butting, aedeagus tapping, female kicks and female head butting was also noted.

195 Overall, 119 mating pairs of *T. granarium* were observed. Males and females
196 that did not engage in any courtship approach or stayed motionless for more than 60
197 min were discarded (n=22 pairs) (Benelli et al. 2016). Therefore, data from 97
198 mating pairs were analysed. For the laterality observations, only females that were
199 free in the middle of the arena when males approached them were evaluated, since
200 those females that were located close to the sides of the arena would affect males'
201 directional approaches, (Romano et al. 2016a,b). The presence of food is not
202 necessary for mating of this species as it has been shown by previous studies (Wojcik
203 1969), thus no kernels or other food sources were provided in the arena in order to
204 avoid any constrain affecting the orientation approach of males. Ninety-seven mating
205 pairs of *T. granarium* were considered for behavioural analysis.

206

207 Data analysis

208

209 Data were analysed by the linear model, separating the effects of the
210 reconnaissance from those of the copulation, as described below:

$$211 \quad y_{ij} = \mu + RS_i + LtS_j + RS_i \times LtS_j + \varepsilon_{ij}$$

212 Where y_{ij} = data observed (recognition time, head wagging, aedeagus tapping time,
213 aedeagus tapping acts, copulation duration, kicks, post-copulation head wagging and
214 whole duration); μ = mean; RS_i = fixed effect of i^{th} recognition side (left, right); LtS_j
215 = fixed effect of j^{th} leg tapping side (left, right); ε_{ij} = casual error.

$$216 \quad y_{ij} = \mu + CLM_i + CA_j + CLM_i \times CA_j + \varepsilon_{ij}$$

217 Where y_{ij} = data observed (copulation duration, kicks and post-copulation head
218 wagging); μ = mean; CLM_i = fixed effect of i^{th} copulation lateral mounting (left,
219 right); CA_j = fixed effect of j^{th} copulation angle (45, 90, 180); ε_{ij} = casual error.

220 The relationship between male recognition laterality, namely left-biased
221 recognition side and leg tapping (LL), left-biased recognition side and right-biased
222 leg tapping (LR), right-biased recognition side and left-biased leg tapping (RL),
223 right-biased recognition side and leg tapping (RR), and the mating success outcome
224 (yes or no) was estimated by a contingency analysis. The same analysis was also
225 adopted to evaluate the relationship between male recognition laterality and
226 mounting attempt laterality, namely left-biased recognition side and leg tapping
227 (LLr), left-biased recognition side and right-biased leg tapping (LRr), right-biased
228 recognition side and left-biased leg tapping (RLr), right-biased recognition side and
229 leg tapping (RRr), left-biased mounting side and leg tapping (LLm), left-biased
230 mounting side and right-biased leg tapping (LRm), right-biased mounting side and
231 left-biased leg tapping (RLm), right-biased mounting side and leg tapping (RRm).

232

233 **Results**

234

235 The courtship and mating behaviour of *T. granarium* is quantified in Figure 1.
236 During preliminary recognition, the *T. granarium* male approached the female from a
237 side and started tapping her with his forelegs and mouthparts (head wagging,
238 hereafter). Then, the male reached the backside of the female and attempted
239 copulation, exploring her with the aedeagus. If the mounting attempt was successful
240 (76% of the observed pairs), the male would not mount on the female's body; it
241 remained on the substrate and his body longitudinal axis formed a typical angle with
242 the female body size. In the majority of cases, the angle was 45° or 90 and rarely
243 reached 180°. After the copulation, the *T. granarium* female actively started to stave
244 off the male, moving her abdomen and performing head-butts or rejection kicks with
245 her hind legs (Figure 1).

246 The presence of four population-level lateralized traits characterizing the
247 courtship and mating behaviour of *T. granarium* is shown in Figure 2. The male
248 courtship and mounting approach, as well as the female post-copulatory kicks were
249 left-biased, while male foreleg tapping acts were right-biased.

250 Left-biased asymmetries in the recognition approach on a given recognition
251 side led to differences in recognition duration, number of head wagging acts and
252 overall duration of the courtship and mating approach (Table 1). Furthermore, right-
253 biased foreleg tapping behaviour by *T. granarium* males was linked to a shorter
254 recognition time (Table 2), while the left-biased mounting approach led to no
255 differences in copulation duration, as well as number of post-copulation head butts
256 and kicks by females (Table 3).

257 Contingency analysis carried out between the parameters male recognition
258 laterality and mating success showed significant differences between the observed
259 and expected values ($\chi^2_{1}=10.63$; $P=0.014$) (Supplementary Online Material Table
260 S1), highlighting that the majority of tested males performed left-biased female
261 recognition (88.2%) achieved 75% of the overall male mating success (Figure 3). In
262 addition, contingency analysis between the parameters male recognition laterality
263 and mounting laterality showed significant differences between the observed and
264 expected values ($\chi^2_{1}=41.34$; $P<0.001$) (Supplementary Online Material Table S2),
265 pointing out that the majority of the males that displayed left-biased recognition
266 phase also showed left-biased mounting approach (Figure 4).

267

268 **Discussion**

269

270 The majority of studies on *T. granarium* reproductive behaviour have been
271 focused on the olfactory cues routing aggregation, as well as male-female
272 interactions. It has been showed that females release an assembling scent starting
273 from their first day after pupal-adult ecdysis (Levinson and Ilan 1970). This scent
274 retains male beetles for variable periods and has an aphrodisiac effect when females
275 are present (Levinson and Ilan 1970). However, the excitation of males when they
276 were previously exposed to the female pheromone exhausted them and became
277 unable to orient towards females (Rahalkar et al. 1972). The female's response to this
278 scent is weaker than that of males and mainly represented by female temporary
279 immobilization. The overall courtship and mating sequence of *T. granarium* can be
280 divided in four main phases, i.e. male preliminary recognition, male copulation

281 attempt, copulation, post-copulatory behaviour. To the best of our knowledge, these
282 findings represent the first quantification of the mating behaviour of this important
283 invasive beetle pest, and can be considered as analogous with recent findings for
284 other major stored-product beetle species (Benelli et al. 2016). However,
285 surprisingly, despite the importance of this species, there is still inadequate
286 information on its mating behaviour. It has been previously noted that *T. granarium*
287 females can mate 3-4 times during their lifespan, and lay more eggs when derived
288 from diapausing larvae (Karnavar 1972). Moreover, *T. granarium* unmated females
289 live longer (20-25 days) than mated females (4-7 days), and no difference in
290 longevity was observed for both unmated and mated males (7-12 days) (Karnavar
291 1972).

292 Concerning behavioural lateralization, this study showed the presence of four
293 population-level lateralized mating traits in *T. granarium*. Indeed, the male
294 recognition and mounting, as well as the female post-copulatory kicks were left-
295 biased, while male foreleg tapping acts were in majority right-biased. We observed
296 that the left-biased asymmetries in the recognition approach led to significant
297 differences in recognition duration, number of head wagging acts, whole mating
298 duration and, most importantly, male mating success. Currently, extremely scarce
299 information is available on behavioural asymmetries in the order Coleoptera.
300 Recently, Benelli et al. (2016) showed population-level lateralization of mating traits
301 in two important stored product pests, the rice weevil, *Sitophilus oryzae* (Coleoptera:
302 Curculionidae), and the confused flour beetle, *Tribolium confusum* (Coleoptera:
303 Tenebrionidae). In agreement with the present study, males of both species showed
304 population-level left-biased copulation approaches of potential mates, achieving

305 higher mating success over right-biased males (Benelli et al. 2016). In addition, in
306 that series of experiments, it was demonstrated that the geographical origin, as well
307 as different mass rearing practices, might affect the existence and magnitude of
308 lateralization. Recently, the impact of geographical origin and rearing media on male
309 mating success and lateralization was studied in three strains of *S. oryzae* (i.e., a
310 Greek strain reared on wheat, a Greek strain reared on maize and a Peruvian strain
311 reared on maize), shedding light on the factors impacting mating behavioural trends
312 (Romano et al. 2016b). In that study, *S. oryzae* males showed right-biased head
313 wagging and left-biased copulation attempts, regardless of the tested strains. Again,
314 in agreement with our study, mating success was higher in males performing left-
315 biased copulation attempts followed by right-biased head wagging behaviour,
316 without significant differences among the tested strains. Therefore, the findings
317 reported above evidenced the common presence of population left-biased copulation
318 attempts in at least three important families of Coleoptera, i.e., Curculionidae,
319 Dermestidae and Tenebrionidae. Although we did not examine different rearing
320 commodities for *T. granarium* in our current study, Romano et al. (2016b) reported
321 that the rearing substratum significantly impacted the mating behavioural parameters
322 of *S. oryzae*. Similar phenomena may also characterize the mating behaviour of *T.*
323 *granarium*, considering in addition the newer reports which show that *T. granarium*
324 enzymatic activity, population development, fecundity, fertility, larval and pupal
325 weight significantly differ among different commodities (Borzoui et al. 2015,
326 Athanassiou et al. 2016). However, further experimental work is needed towards this
327 hypothesis. In general, courtship of *T. granarium* was shorter than that observed in
328 the case of *S. oryzae* and *T. confusum*, but mating success was overall at the same

329 level (Benelli et al., 2016, Romano et al. 2016b). In contrast with *S. oryzae* and *T.*
330 *confusum*, adults of *T. granarium* are short-lived, and this characteristic may be
331 related with shorter copulation duration. For another short-lived stored product
332 species at the adult stage, the cowpea seed beetle, *Callosobruchus maculatus*
333 (Coleoptera: Bruchidae) mating was also short (der Hollander and Gwynne 2009).

334 Overall, results reported here represent a rather unique example of four
335 subsequent mating traits lateralized at population-level, which influence the male
336 mating success. In light of our findings, lateralization of *T. granarium* is
337 quantitatively different than that for other major stored product beetle species
338 (Romano et al. 2016b, Benelli et al. 2016). Based on the results of the present study,
339 it becomes evident that *T. granarium* mating is generally faster than that for other
340 beetle species that share the same environment, and this characteristic may lead
341 rapidly to increased progeny production when the conditions prevailing are suitable.
342 This may be related with the fact that adults of *T. granarium* are short lived, in
343 contrast with the majority of other key stored product beetle species. As a result,
344 mating and oviposition has to be completed in a short interval, which may lead to
345 short intervals that this species can build with ease high population densities. A
346 better understanding of mating asymmetries and courtship plasticity variables in *T.*
347 *granarium* can contribute on the utilization of some of these cues for beetle control,
348 i.e., by inducing parameters that can moderate mating success. Successful paradigms
349 of this perception have been documented in the case of fruit flies, i.e., in Sterilized
350 Insect Technique (SIT) programs, where adults should have specific mating
351 characteristics (Benelli et al. 2015a). Further experimental work is required to

352 investigate the potential utilization and practical moderation of these parameters for
353 *T. granarium*, under the basis of an area-wide management of this invasive species.

354

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356

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366 and analysis, decision to publish, or preparation of the manuscript.

367

368 **Compliance with ethical standards**

369

370 All applicable international and national guidelines for the care and use of
371 animals were followed. All procedures performed in studies involving animals were
372 in accordance with the ethical standards of the institution or practice at which the
373 studies were conducted.

374

375 **Conflict of interest**

376

377 The authors declare no conflicts of interest.

378

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380

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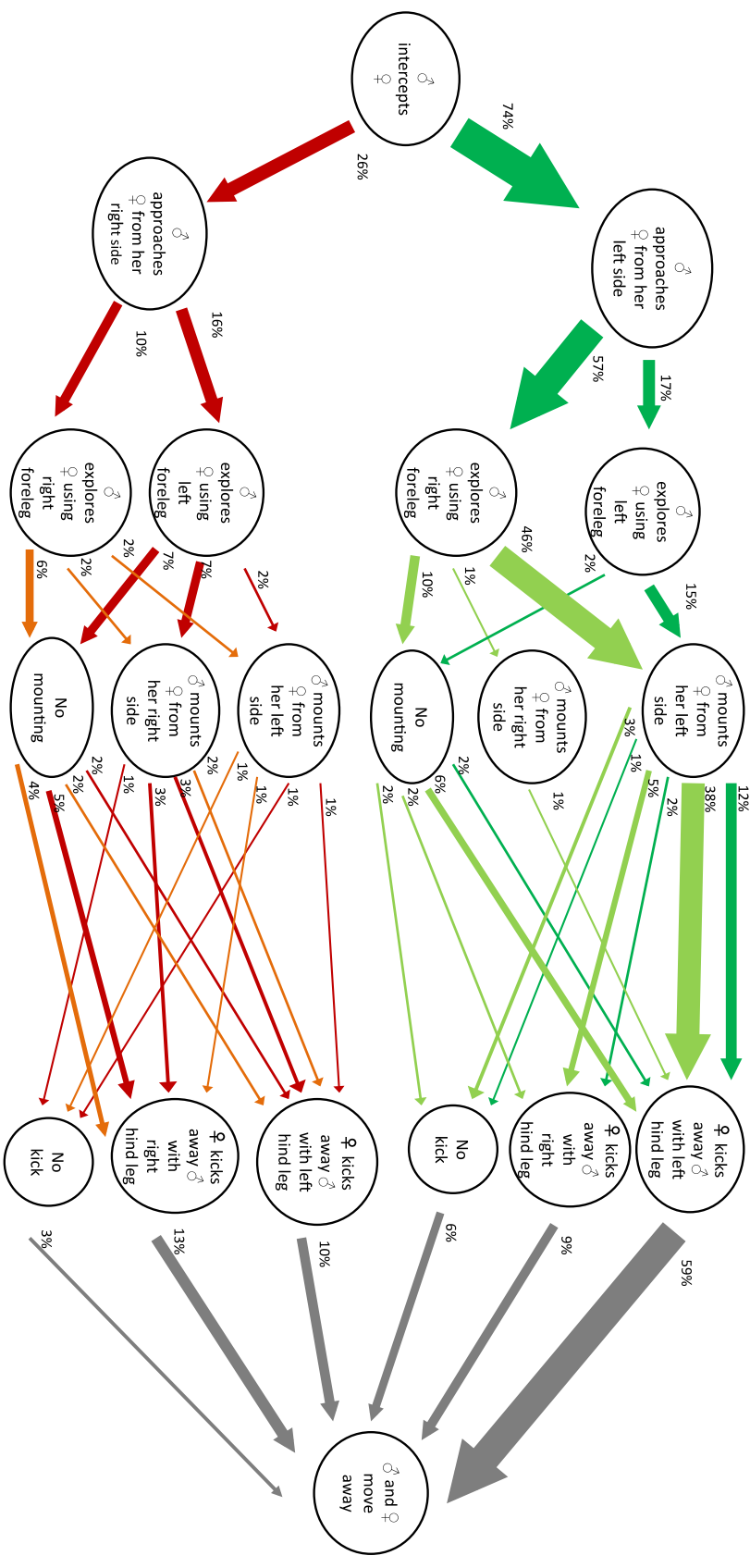
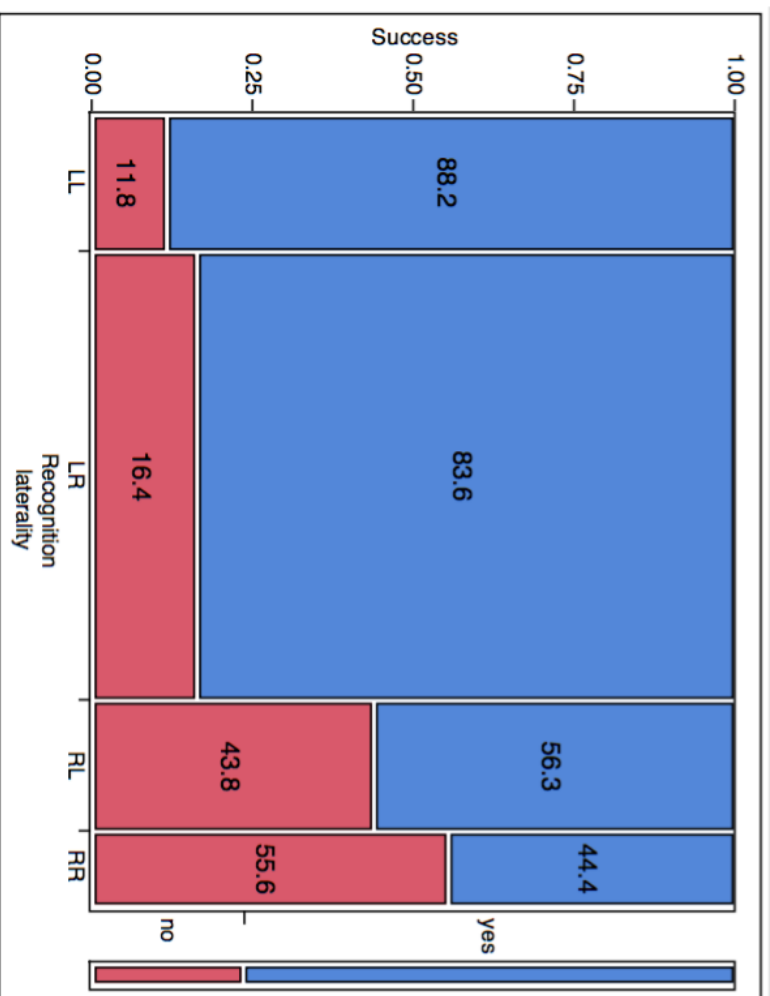


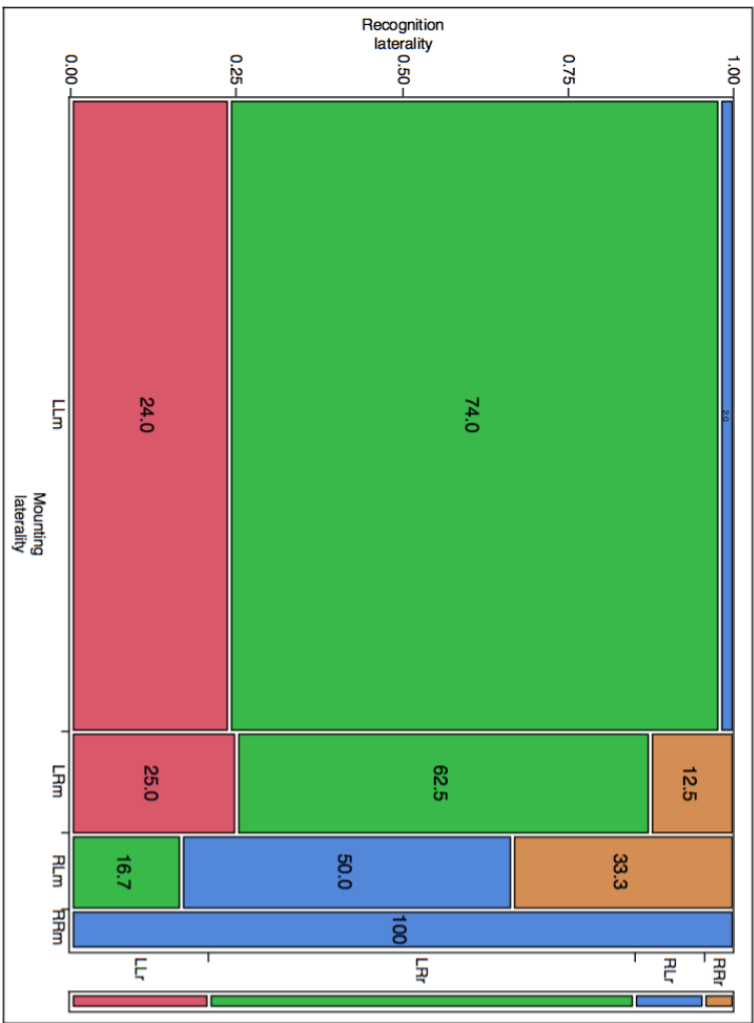
Figure 2. Lateralized traits characterizing the courtship and mating behaviour of *Trogoderma granarium*. The thickness of each arrow indicates the proportion of beetles displaying each behaviour (n=97 analyzed pairs).

Figure 3. Contingency analysis between male recognition laterality and mating success of *Trogoderma granarium*. Recognition laterality is reported on x-axis, while the outcomes of copulation (i.e. success or unsuccessful) are reported on y-axis. The horizontal width of each recognition laterality indicates the relative abundance of a given lateralized combination over the total number of tested beetles. The bar on the right represents the relative abundance of copulation outcomes over the total number of tested beetles. The number in the each box indicates the percentage of copulation outcomes within each lateralized recognition combination.



LL = left-biased recognition side and leg tapping
LR = left-biased recognition side and right-biased leg tapping
RL = right-biased recognition side and left-biased leg tapping
RR = right-biased recognition side and leg tapping
yes = the male achieved mating success
no = the male did not achieve mating success

Figure 4. Contingency analysis between male recognition laterality and mounting laterality in *Trogoderma granarium*. Mounting laterality is reported on x-axis, while recognition laterality is reported on y axis. The horizontal width of each mounting laterality indicates the relative abundance of a given lateralized combination over the total number of tested beetles. The bar on the right indicates the relative abundance of recognition laterality over the total number of tested beetles. The number in each box indicates the percentage of different lateralized recognition within each lateralized mounting combination.



Llr = left-biased recognition side and leg tapping
LRr = left-biased recognition side and right-biased leg tapping
RLr = right-biased recognition side and left-biased leg tapping
RRr = right-biased recognition side and leg tapping
Llm = left-biased mounting side and leg tapping
LRm = left-biased mounting side and right-biased leg tapping
RLm = right-biased mounting side and left-biased leg tapping
RRm = right-biased mounting side and leg tapping

Table 1. Effect of the recognition side on the main mating traits of *Trogoderma granarium*; the asterisk indicates significant differences (general linear model, $P < 0.05$).

Parameter	Left side	Right side	P-value
Recognition time (s)	24.82 ± 1.31	17.77 ± 2.63	0.019 *
Head wagging (n)	4.47 ± 0.41	1.61 ± 0.84	0.003 *
Aedeagus tapping time (s)	5.87 ± 0.61	6.07 ± 1.23	0.885 n.s.
Aedeagus tapping acts (n)	3.57 ± 0.39	4.34 ± 0.81	0.394 n.s.
Copulation (s)	65.83 ± 2.03	63.15 ± 4.09	0.561 n.s.
Kicks (n)	3.05 ± 0.36	2.51 ± 0.72	0.506 n.s.
Post-copulation head-butts (n)	1.31 ± 0.26	0.63 ± 0.53	0.256 n.s.
Whole duration (s)	102.15 ± 2.63	88.75 ± 5.32	0.027 *

n.s. = not significant

Table 2. Effect of the leg tapping side on the main mating traits of *Trogoderma granarium*; the asterisk indicates significant differences (general linear model, $P < 0.05$).

Parameter	Left side	Right side	P-value
Recognition time (s)	24.48 ± 1.85	18.11 ± 2.29	0.034 *
Head wagging (n)	3.78 ± 0.59	2.30 ± 0.73	0.119 n.s.
Aedeagus tapping time (s)	4.98 ± 0.86	6.96 ± 1.07	0.155 n.s.
Aedeagus tapping acts (n)	3.22 ± 0.56	4.70 ± 0.69	0.104 n.s.
Copulation (s)	64.27 ± 2.87	64.70 ± 3.55	0.926 n.s.
Kicks (n)	2.68 ± 0.50	2.87 ± 0.62	0.817 n.s.
Post-copulation head-butts (n)	0.89 ± 0.37	1.06 ± 0.46	0.766 n.s.
Whole duration (s)	98.10 ± 3.73	92.80 ± 4.62	0.299 n.s.

n.s. = not significant

Table 3. Effect of copulation side on the main mating traits of *Trogoderma granarium* (general linear model, $P > 0.05$).

Parameter	Left side	Right side	P-value
Copulation (s)	67.34 ± 3.43	58.43 ± 4.27	0.187 n.s.
Kicks (n)	3.46 ± 0.57	2.98 ± 0.91	0.656 n.s.
Post-copulation head-butts (n)	0.89 ± 0.30	0.69 ± 0.71	0.809 n.s.

n.s. = not significant