The role of lateralization in mating behavior and mating success of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

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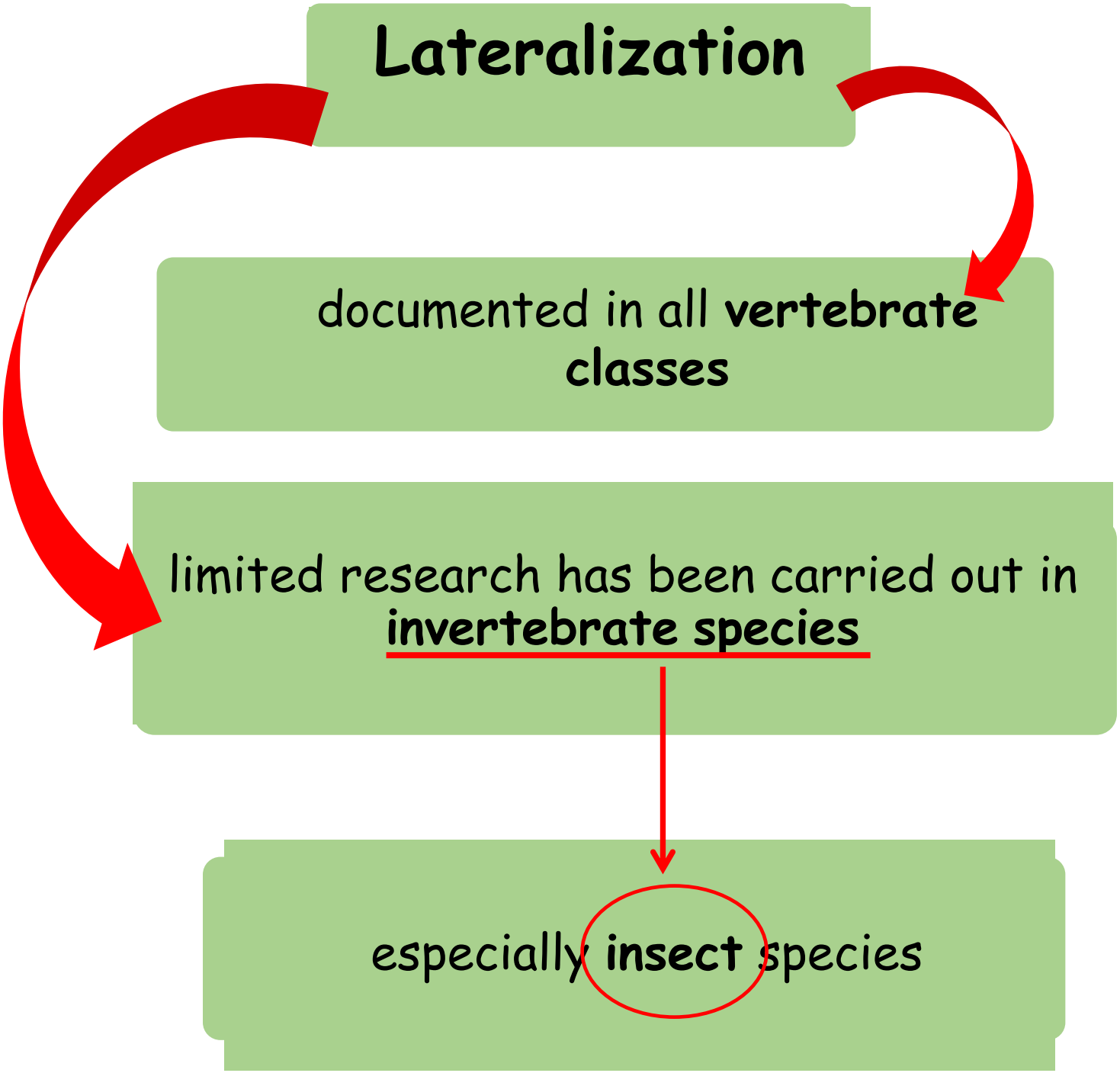
CA22140 - Improved Knowledge Transfer for Sustainable Insect Breeding (Insect-IMP)

INSECT-IMP

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

Lateralization: left and right asymmetries in brain and behaviour

enhance brain efficiency in cognitive tasks involving both hemispheres concurrently but differently


Lateralization
documented in all **vertebrate classes**
limited research has been carried out in **invertebrate species**
especially **insect** species

Research lateralization in insects:

Bees >

Diptera:
Ceratitis capitata
Bactrocera oleae
Calliphora vomitoria
Lucilia sericata
Aedes albopictus
Culex pipiens

Dermaptera:
Labidura riparia
Euborellia plebeja
Nala lividipes
N. nepalensis

Orthoptera:
Locusta migratoria
Schistocerca gregaria

>

Stored-product

Coleoptera

Tribolium confusum
Sitophilus oryzae
Trogoderma granarium
Rhyzopertha dominica
Oryzaephilus surinamensis
Cryptolestes ferrugineus
Prostephanus truncatus
Alphitobius diaperinus

Aim of the present study

- impact of lateralization on mating succe

Tenebrio molitor

(Coleoptera: Tenebrionidae)

Materials and methods

Insects and sex recognition

- **Virgin mature** males and females > 5 d old

- **Sex identification** → pupal stage (Bhattacharya et al., 1970)
  → separately transferred

Petri dishes (diameter 50 mm, height 10 mm)

30°C and 65% RH → in continuous darkness

emergence of adults

kept in the same plastic cups or Petri dishes

Materials and methods

Behavioral observations

- **Petri dish arenas** (80 mm diameter and 10 mm height) → surrounded with wall of white filter paper → avoid visual cues

- **Preliminary observations** → mating pairs → highly sexually active → 11.00 to 19.00

Behavioral observations of mating pairs

Natural light

27 °C and 60% RH

Materials and methods

Behavioral observations

a virgin male and a virgin female were transferred into the testing arena → observed for 60 min, or until the end of the sexual interaction

* recorded the mating and laterality behavior → *Tenebrio molitor*

following phases:

(i) mate recognition (time spent by ♂ to detect ♀)

(ii) Precopula (time spent by ♂ for courtship, including antennal tapping, rubbing her elytra with his forelegs and middle legs, attempting to mount the ♀ until genital contact)

(iii) copula (from the ♂ insertion of the aedeagus into the genital chamber of ♀ until genital disengagement

(iv) duration whole mating sequence

which side of female's body was preferred by male for mounting

Materials and methods

1) males and females remained motionless
2) did not engage in any courtship activity
3) the females were near the walls

Tenebrio molitor

299 mating pairs

113 pairs

186 pairs → analyzed

Fig. 1. Quantitative analysis of *Tenebrio molitor* courtship and mating behavior. Two distinct mate recognition approaches characterizing this species are indicated with black and blue arrows. The thickness of each arrow indicates the proportion of beetles displaying each behavior (*n* = 186 mating pairs).

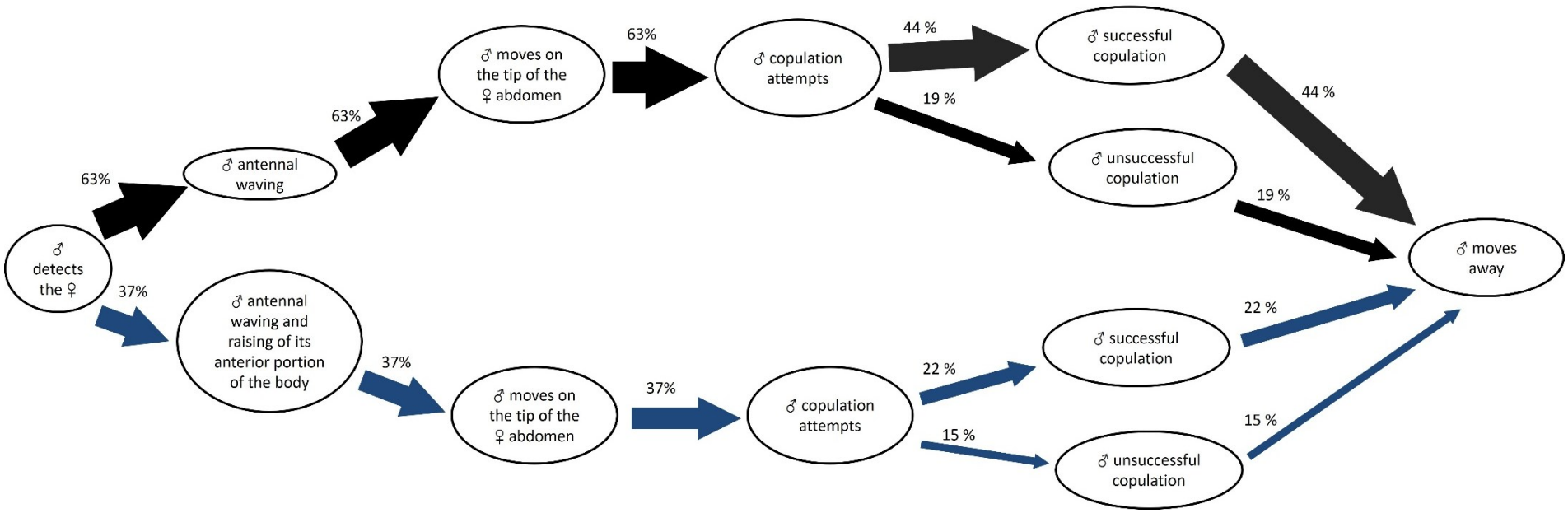


Fig. 2. Flow chart quantifying lateralized mounting in ***Tenebrio molitor***. **Black**, **blue**, **green**, and **red** arrows indicate the body side of females (**left**, **right**, **back**, and **front side**, respectively) preferred by males. The thickness of each arrow indicates the proportion of beetles displaying each behavior (*n* = 186 mating pairs).

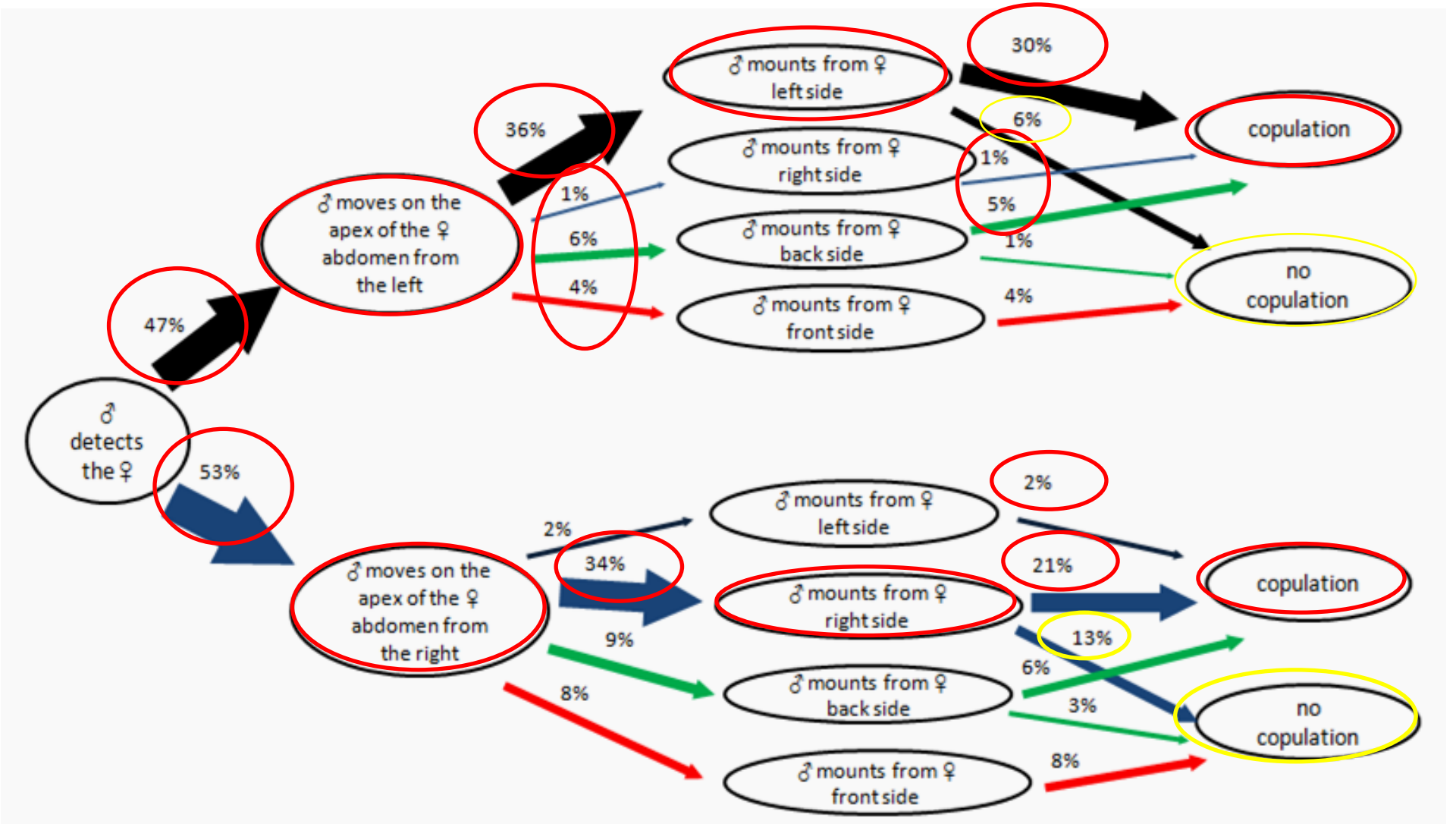


Fig. 3. *Overall quantitative analysis of the lateralized courtship and mating behavior of T. molitor.* Male laterality bias during mate recognition and mounting attempts is showed by red, green, orange and purple arrows indicate the body side of females (left, right, back, and front side, respectively). The thickness of each arrow indicates the proportion of beetles displaying each behavior (*n* = 186 mating pairs).

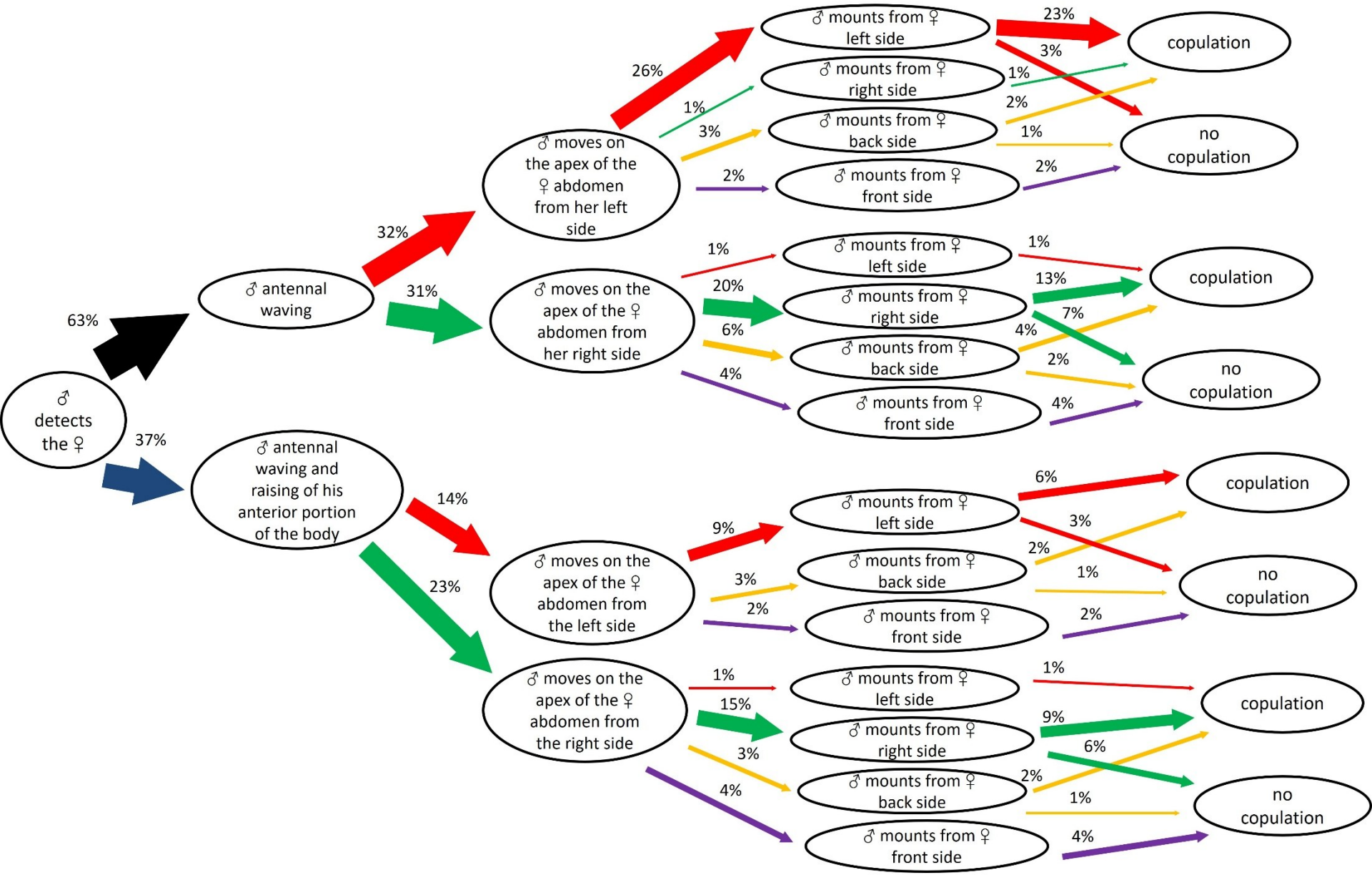


Table 1. Effect of the recognition side bias on the duration of the *T. molitor* main mating traits. Values are means followed by standard errors (SE). Asterisks within each column indicate significant differences; Steel-Dwass test at 0.05. Where no asterisks exist, no significant differences were noted.

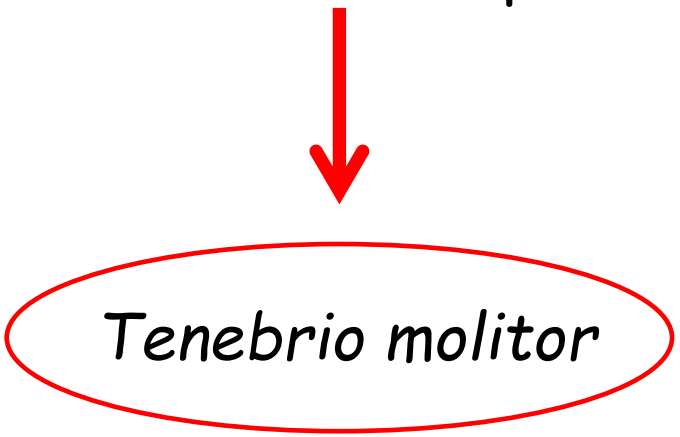
Laterality bias	Mate recognition (s)	Precopula		Copula (s)	Whole mating sequence (s)
		Antennal tapping (s)	Rubbing behavior (s)		
Left-biased	10.7 ± 0.6	12.6 ± 0.8	16.4 ± 1.1	86.4 ± 3.7	126.1 ± 3.9
Right-biased	12.2 ± 0.6*	14.8 ± 0.8*	18.6 ± 1.2*	94.1 ± 5.8	139.6 ± 5.9*
χ^2	4.26	6.73	4.29	0.99	3.87
df	1	1	1	1	1
P	0.0391	0.0095	0.0383	0.3193	0.0493
Tested beetles (*n* = left + right-biased)	65 + 58 = 123	65 + 58 = 123	65 + 58 = 123	65 + 58 = 123	65 + 58 = 123

Table 2. Effect of the mounting side on the duration of the *T. molitor* main mating traits. Values are means followed by standard errors (SE). Within each column, means followed by the same letter are not significantly different; Steel-Dwass test at 0.05. Where no letters exist, no significant differences were noted.

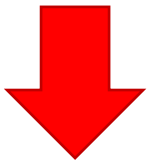
Side bias	Mate recognition (s)	Precopula		Copula (s)	Whole mating sequence (s)
		Antennal tapping (s)	Rubbing behavior (s)		
Left-biased	10.5 ± 0.6	12.8 ± 0.8	15.5 ± 0.9b	84.5 ± 3.9	123.2 ± 4.0b
Right-biased	12.0 ± 0.6	14.7 ± 0.9	18.9 ± 1.5a	92.7 ± 6.9	138.3 ± 7.0a
Backside	12.6 ± 1.1	13.7 ± 1.2	19.4 ± 2.2a	98.5 ± 7.3	144.1 ± 7.4a
χ^2	4.96	3.60	6.05	3.61	7.89
df	2	2	2	2	2
P	0.0839	0.1678	0.0486	0.1648	0.0193
Tested beetles (*n* = left + right + back-biased)	56 + 45 + 22 = 123	56 + 45 + 22 = 123	56 + 45 + 22 = 123	56 + 45 + 22 = 123	56 + 45 + 22 = 123

Conclusions

- **basic knowledge** on the role of behavioral asymmetries in the courtship and mating



Left-biased approach and **copulation attempts**



higher mating success over left-biased males → **laterality**
chiefly contributed to the male mating success

Conclusions

- Previous behavioural studies of stored-product insects → **left-biased trend** → elevate proportions of mating success

Left-biased males

Tribolium confusum
Tribolium castaneum
Sitophilus oryzae
Trogoderma granarium
Cryptolestes ferrugineus

→ higher mating success

Right-biased males

Rhyzopertha dominica
Oryzaephilus surinamensis
Prostephanus truncatus
Alphitobius diaperinus

→ higher mating success

Conclusions

- behavioral lateralization plays a significant role on the duration of the main mating traits
  - duration of **mate recognition**, **precopula** (i.e., antennal tapping and rubbing behavior), and the **whole courtship and mating sequence** were significantly lower in left-biased over right-biased
  - the male preference for mounting side significantly affected the duration of the **rubbing behavior** (precopula) and the **whole mating sequence**

Conclusions

- **useful tool:**

  - understand the basic biological traits of *Tenebrio molitor*

  - optimize mass-rearing techniques

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Research

Stored-Product

Behavioral Asymmetries Affecting Male Mating Success in *Tenebrio molitor* (Coleoptera: Tenebrionidae), an Important Edible Species

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Abstract

The yellow mealworm, *Tenebrio molitor* L., is one of the most significant insect species of economic importance for producing protein-rich food and feed. The larvae are a promising fishmeal substitute for fish feed, and a good alternative source for human nutrition. In this study, the effect of behavioral asymmetries on male mating success of *T. molitor* was evaluated. Males performing antennal waving (63%) when detecting a female approached the apex of the female abdomen in a comparable manner from both sides (32% from left side vs 31% from right side). Fewer males showed antennal waving and also raised the anterior part of their body (37%) during mate recognition; 14% of them approached on the apex of female abdomen from the left side, and 23% of them approached from the right side of female body. The duration of mate recognition, antennal tapping, rubbing behavior, and the whole mating sequence of males that moved on the apex of the abdomen from the left side of females was significantly lower over that of males approaching from the right side (10.7, 12.6, 16.4, and 126.1 s for left-biased males vs 12.2, 14.8, 18.6, and 139.6 s for right-biased males, respectively). Concerning mounting side, left-biased males showed a shorter duration of rubbing behavior and the whole mating sequence (15.5 and 123.2 s) over right-biased (18.9 and 138.3 s) and backside approaching males (19.4 and 144.1 s). The duration of mate recognition, antennal tapping, and copula was not affected by the presence of a male laterality bias. Overall, this study sheds light on how laterality affects mating traits and the male success of this important edible insect species.

Key words: yellow mealworm, insect-based foods, lateralization, mass-rearing, stored-product beetles

Thank you for your attention