Relationship between weapon size and six key behavioural and physiological traits in males of the European earwig

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SUMMARY

In many animals, male weapons are large and extravagant morphological structures that typically enhance fighting ability and reproductive success. It is generally assumed that growing and carrying large weapons is costly, thus only males in the best condition can afford it. In the European earwig, males carry weapons in the form of forceps-like cerci, which can vary widely in size within populations. While long forceps appear to increase male's access to females, it is unknown whether it also correlates with other important male life-history traits. This information is important, however, in determining the potential reliability of forceps length as an indicator of male quality and the stability of this signalling system. Here, we tested whether forceps length is associated with six important behavioural and physiological traits in males of the European earwig. We sampled hundreds of males from two populations, selected 60 males with the longest and shortest forceps from each population, and then measured locomotor performance, boldness, aggregation behaviour, survival under harsh conditions, sperm storage, and survival after pathogen exposure. Contrary to our predictions, we detected no main association between forceps length and the traits measured. This lack of association was consistent between the two populations, although there were population-specific levels of boldness, aggregation and survival in harsh conditions (for long-forceps males only). Overall, these results challenge our current understanding of the function and quality signal of forceps length in this species and raise questions about the evolutionary drivers that could explain the maintenance of weapon size diversity within and between populations.

Keywords: Behaviour; Insect; Metarhizium; Ornament; Sexual selection; Weapons

INTRODUCTION

Animal reproduction often requires males to engage in physical competition and courtship to attract females in search of mates (Davies et al., 2012). From vertebrates to arthropods, these two needs have often led to the evolution of male weapons and ornaments through sexual selection (Emlen, 2008; Goldberg et al., 2019; McCullough et al., 2016). These weapons and ornaments are typically large and extravagant morphological structures that can grow on different parts of the male's body, take a variety of forms (such as antlers, horns, spurs, fangs and tusks), and work to enhance the male's fighting ability and/or attractiveness to females (Emlen, 2008). Textbook examples of this enhancement can be found in the white-tailed deer *Odocoileus virginianus*, where males growing the largest antlers are more likely to win fights with other males and have higher annual breeding success (Newbolt et al., 2017), and in the stalk-eyed flies *Cyrtodiopsis whitei* and *C. dahnanni*, where females prefer to mate with males exhibiting the longest eye stalks (Wilkinson et al., 1998).

However, not all males display extravagant weapons or ornaments (Emlen, 2008; Goldberg et al., 2019; McCullough et al., 2016), as the development and maintenance of these sexually selected traits often comes at a cost to males. This cost can arise from the fact that carrying heavy, bulky weapons (or ornaments) makes males less mobile and more visible to both predators and prey (Oufiero & Garland, 2007). For example, males with experimentally-enlarged wing spots have lower survival rates due to increased conspicuity to both visually orienting predators and visually orienting prey in the rubyspot damselfly *Hetaerina americana* (Grether, 1997). The cost of carrying large morphological structures can also arise because it may impose investment trade-offs with physiological functions ranging from metabolism to muscle development and spermatogenesis,

56 which can be crucial for male fitness and survival (Emlen, 2001). For example, males with the
57 largest hind leg weapon pay the highest resting metabolic rate and energy costs in the Hemiptera
58 *Leptoscelis tricolor* (Somjee et al., 2018). Similarly, growing large mandibles come with low flight-
59 muscle mass in the stag beetle *Cyclommatus metallifer* (Mills et al., 2016), and males who invest
60 in extravagant sexual displays show a more rapid decline in spermatogenesis than males who
61 invest less in these displays in the houbara bustard *Chlamydotis undulata* (Preston et al., 2011). As
62 a result, only the males in the very best condition are expected to possibly afford the development
63 and maintenance of extravagant weapons and ornaments (Otte & Stayman, 1979; Emlen &
64 Nijhout, 2000). Determining the reliability of these morphological structures as indicators of the
65 male quality is therefore crucial to understanding the stability of these signalling systems
66 (Berglund et al., 1996).

67 Earwigs (Dermaptera) are textbook examples of insects with males displaying a sexually
68 selected weapon. In this taxonomical group, females have relatively short, straight and hardened
69 forceps-like cerci typically used to defend the clutch against predators (Meunier, 2024), whereas
70 males display elongated, curved and hardened forceps-like cerci (hereafter referred to as forceps)
71 involved in mating courtship and fights against other males (Briceño & Eberhard, 1995; Kamimura,
72 2014; Walker & Fell, 2001). There are several lines of evidence to suggest that long forceps provide
73 males with benefits in terms of mating success (Eberhard & Gutierrez, 1991; Kamimura, 2014;
74 Tomkins & Brown, 2004). For example, males with long forceps are more likely to win fights with
75 other males by squeezing them between their cerci, and thus gain better access to females in the
76 toothed earwig *Vostox apicedentatus* (Moore & Wilson, 1993). In the European earwig *Forficula*
77 *auricularia,* male forceps are also used in male-male contests as a weapon to deter competitors

78 prior to mating (Styrsky & Rhein, 1999) or to interrupt mating individuals by non-copulating males
79 (Forslund, 2000, 2003; Walker & Fell, 2001). Long-forceps males are also generally more
80 aggressive, more readily accepted by females for mating and copulate longer compared to short-
81 forceps males (reviewed in Kamimura, 2014). Although forceps are involved in male courtship
82 (Walker & Fell, 2001), females do not seem to select their mate exclusively on the basis of forceps
83 length (Forslund, 2000, 2003; Radesäter & Halldórsdóttir, 1993; Walker & Fell, 2001).

84 While the relationship between male forceps length and mating success has been well
85 documented in earwigs (reviewed in Kamimura, 2014), it remains unclear whether having long or
86 short forceps is associated with other important life-history traits in these males. However, this is
87 important information to determine the potential reliability of forceps length as an indicator of
88 male quality and the stability of this possible signalling system. The very few studies that have
89 addressed this question focused on male immunity in the European earwig *F. auricularia* (Körner
90 et al., 2017; Rantala et al., 2007). On the one hand, they show that males with long forceps have
91 lower basal levels of certain components of the immune system, such as lysozyme activity and
92 hemocyte concentrations. This suggests that having long forceps comes with an immune cost for
93 males. On the other hand, they show no association between forceps length and other
94 components of the immune response, such as encapsulation rate and phenoloxidase activity.
95 Taken together, these findings suggest that the immune costs of having long forceps may be
96 relatively limited and raise the question of whether other important life-history traits can be
97 associated with forceps length.

98 Here, we investigated whether long and short forceps are associated with six important
99 behavioural and physiological traits in males of the European earwig. We sampled hundreds of

100 males in two natural populations of earwigs separated by 400 km and in each population, we
101 selected the 30 males with the longest and the 30 males with the shortest forceps. We then
102 measured their level of expression of three important behaviours, their survival rate in two distinct
103 harsh conditions and their sperm quantity. The first behaviour was their locomotor performance
104 (Cheutin et al., 2024), which reflects the ability of males to walk long distances (to forage, hide or
105 find a mate) while carrying long and heavy (or short and light) appendages. The second behaviour
106 was their likelihood to flee after a physical disturbance (i.e., boldness), which shows how males
107 react when disturbed by a predator attack (Thesing et al., 2015). The third behaviour was their
108 propensity to aggregate with conspecifics. This is an important parameter in this gregarious
109 species, as adults typically live in groups of up to several hundred individuals and social isolation
110 can have detrimental effects on their physiology (Kohlmeier et al., 2016; Van Meyel & Meunier,
111 2022). We also measured their survival rate in a harsh condition where they were isolated with no
112 access to a food source for 31 days, and then their survival rate after exposure to the common
113 entomopathogenic fungus *Metarhizium brunneum* (Vogelweith et al., 2017). Finally, we measured
114 the level of sperm storage in seminal vesicles of each male, a parameter that is often important in
115 the context of male-male competition for mating (Shuker & Simmons, 2014). If forceps length is a
116 reliable signal of good quality, we predict that long forceps males would 1) have higher locomotor
117 performance, 2) be bolder and thus less likely to flee after a simulated attack, 3) be more social
118 and thus more likely to aggregate with conspecifics, 4) be more resilient to harsh environmental
119 conditions and thus have a higher survival rate after 31 days of starvation, and/or would 5) have
120 higher immune defences and thus survive better after a pathogen infection than short-forceps
121 males. We also predict that 6) long-forceps males would produce more sperm and contain more

sperm in their seminal vesicles than short forceps males. This last prediction would be consistent with the longer duration of copulation reported for the long-forceps males (Kamimura, 2014), even if this longer duration may also reflect other male mating strategies, such as mate guarding.

MATERIAL AND METHODS

Animal rearing and selection

In July 2022, we field sampled 3000 males and females in a cultivated peach orchard near Valence, France (Lat 44.9772790, Long 4.9286990) and 400 males and females in a non-cultivated area at the edge of a forest near Cinais, France (Lat 47.1606970, Long 0.1763663). All these individuals belong to *Forficula auricularia* Linneaus, 1758, also called *Forficula auricularia* clade A (González-Miguéns et al., 2020). Immediately after field sampling, we distributed them by population of origin into 50 plastic terrariums (37 × 22 × 25 cm, balanced sex ratio) to homogenize nutrition, habitation, and access to mates for the males (Körner et al., 2017). Three months later (i.e., at the end of the reproductive season), we removed the females from all the terrariums to mimic their natural dispersal. One month later, for each population, we visually selected the 30 males with the longest forceps and the 30 males with the shortest forceps (Körner et al., 2017) and isolated them in individual Petri dishes (diameter 5 cm) for later use in behavioural and physiological measurements (A timeline of the experimental design and the detailed sample sizes can be found in Figure 1). Two days after isolation, we confirmed the robustness of our two forceps categories by measuring the mean of the left and right outer forceps of the 120 males. These measurements were done to the nearest 0.001 mm using the Leica Application Suite 4.5 software (Leica

143 Microsystems, Wetzlar, Germany) on pictures taken under a binocular scope (Leica, MZ 12.5).
144 These measurements confirmed that there was no overlap between the two male categories
145 (Figure 2). All the remaining males and females that were not used in this experiment were
146 involved in other experiments that are not presented here.

147 From field sampling to isolation, we fed animals with artificial food containing mostly
148 pollen, carrots, and cat food (see details in Kramer et al. 2015). During these three months, we
149 offered this food *ad libitum* to homogenise the nutritional condition of the males when we started
150 our measurements. We then kept the isolated males without access to food from the day they
151 were isolated until 31 days after their isolation (Figure 1) to test whether resistance to both
152 starvation and social isolation (i.e., harsh environmental conditions) were population and/or
153 forceps-length specific, while ensuring that good rearing conditions did not mask any potential
154 investment trade-offs between forceps length and other life history traits. We then provided food
155 to males after day 31 to measure their survival following pathogen exposure. We kept all animals
156 on a 12h:12h light:dark schedule at 18°C, and both the terrarium and Petri dishes contained a layer
157 of moist sand.

158

159 ## Behavioural measurements

160 From the 5th to the 24th day after isolation, we measured three behaviours in the 120 isolated
161 males: the locomotor performance, the likelihood of fleeing after a physical disturbance, and the
162 level of aggregation. All these measurements followed standard protocols for earwigs (Honorio et
163 al., 2023; Merleau et al., 2022).

First, we measured male's locomotor performance 5 days after isolation. On that day, we transferred each male to a 3D-printed circular arena (Open field; diameter = 8 cm, height 0.4 cm) with a cover made of glass, placed on an infrared table and kept in complete darkness. We then video recorded males' locomotion for 20 minutes (Camera: BASLER BCA 1300, Germany; Media Recorder v4.0, Noldus Information Systems, Netherland) and subsequently analyzed the resulting videos with the software Ethovision XT 16 (Noldus Information Systems, Netherland). We defined the locomotor performance of each male as the total distance he walked (in cm) during the entire recording (Honorio et al., 2023; Merleau et al., 2022).

Second, we measured the likelihood of fleeing after a physical disturbance 10 days after isolation. On that day, we carefully opened each Petri dish, pricked the male on the pronotum with a glass capillary and then recorded whether or not the male's first reaction was to move more than one body length away from its initial position (i.e., flee).

Finally, we measured the level of aggregation of each male 24 days after isolation. We placed each male in a 3D-printed arena used in Van Meyel & Meunier, 2022, consisting of four linearly aligned circular chambers (diameter 4 cm). Three of the chambers were connected by 0.5 cm wide corridors allowing earwigs to move between chambers. The width of the corridor connecting to the fourth (outer) chamber was reduced to 0.15 cm, which prevented earwig movement while allowing the circulation of odours and antennal contacts between individuals on both sides (Van Meyel & Meunier, 2022). We started the experiment by placing two naive males and one female from the same population (but not involved in any other experiment or measurement) in this isolation chamber, and the tested male in one of the connected chambers. We recorded whether the tested male was in the chamber next to the group of conspecifics (yes

or no) and repeated this measurement by taking pictures every hour for 48 hours using infrared cameras and the software Pylon Viewer v5.1.0 (Basler©, Ahrensburg, Germany). For each tested male, we thus obtained an aggregation score ranging from 0 to 48, which was defined as the total number of pictures in which a male was in the chamber next to the group of conspecifics. After the 48h of aggregation test, we returned each male to its Petri dish until its use for subsequent measurements (see below). For ease of handling, all manipulated earwigs were anaesthetised with CO_2 during the set-up of this last measurement.

Sperm storage measurement

Of the 91 males still alive on day 31 after isolation (Figure 1), we used a random subset of 39 to measure sperm storage as the number of sperm present in their seminal vesicles (Figure 1). This counting occurred about two months after the males were separated from the females, which is probably long enough for the males to rebuild their sperm reserves, regardless of their previous mating rate. We counted sperm numbers following the protocol detailed in Damiens et al. (2002). In brief, we dissected each male under a dissecting microscope, placed their seminal vesicles on a slide with 15 µL of 1x Phosphate Buffered Saline and then pierced it to release all the sperm. We subsequently dried the plate on a heating block, after which the smear of sperm was sealed with a 70% ethanol solution and allowed to dry again at ambient temperature. The slides were then stored at 3°C. Two days later, we deposited 20µL of DAPI dye (concentration = 10 µmol/L) on the smear, covered it with a small piece of plastic wrap to allow the dye to infiltrate the cells while not drying out, and 10 minutes later, we replaced the plastic wrap with a glass slide cover. The slide was then viewed under the microscope at 20x magnification, and we took pictures of 5 different

fields of the smear. Using an Olympus micrometre calibration slide and the software Image J (Schneider et al., 2012), we then counted the number of spermatozoa in all 5 fields for each male. Finally, we used all these numbers to calculate the number of spermatozoa per millimetre square and multiplied this number by the total area of the smear to obtain the total number of sperm per male. It should be noted that sperm storage was measured in males that survived 31 days in isolation without access to food, so that this value represents the sperm storage of individuals best adapted to these two stressful conditions.

Survival in harsh environments and after exposure to pathogens

We measured male survival under two types of harsh conditions. The first type of harsh condition was the absence of any food source (starvation) combined with social isolation, which is known to have detrimental effects on this gregarious species (Kohlmeier et al., 2016; Van Meyel & Meunier, 2022). We assessed the male survival rate under these conditions by recording whether each of the 120 males tested was still alive on day 31 after isolation (Figure 1).

The second type of harsh condition was exposure to pathogens. We measured survival rate after pathogen exposure in the 52 males that were still alive on day 31 and were not used to measure sperm storage (Figure 1). We exposed each male to spores of the entomopathogenic fungus *Metharizium brunneum* (formerly *M. anisopliae*). This fungus is a natural and lethal pathogen of *F. auricularia* (Arcila & Meunier, 2020; Coulm & Meunier, 2021; Günther & Herter, 1974). The infection followed a standard protocol detailed in Kohlmeier et al. (2016). In brief, we immersed each male in an Eppendorf tube containing 1.5 mL of a conidiospore suspension of *M.*

brunneum diluted in 0.05% Tween 20 (Sigma P-1379) at a concentration of 10^6 spores/mL. We then gently swirled the tube from side to side for 4 seconds, removed the male and placed it back in its original Petri dish with standard food (see above) that was changed twice a week. We then kept the infected males at 20°C on a 12:12 light:dark schedule. We checked them daily for mortality over the course of 45 days. As with sperm storage, survival after pathogen exposure was measured in males that survived 31 days in isolation without access to food, so that it represents the survival rate of individuals best adapted to two these stressful conditions.

Statistical analyses

We conducted all statistical analyses using the software R v4.3.2 (https://www.r-project.org/) loaded with the packages *DHARMa* (Hartig, 2020), *car* (Fox & Weisberg, 2019), *survival* (Therneau, 2020) and *emmeans* (Lenth, 2022). We analysed locomotor performance, sperm count and aggregation score using three general linear models (*lm* in R), while we analysed the likelihood to flee (yes or no) and whether males were dead 31 days after isolation (yes or no) using two generalized linear model (*glm* in R) with binomial error distributions. Finally, we analysed the survival rate of the pathogen-exposed males using a Cox proportional hazard regression model allowing for censored data (*Coxph* in R), i.e., males still alive 45 days after exposure to the pathogen. In these six models, we entered the type of forceps length (long or short), the population of origin of the males (Cinais or Valence) and the interaction between these two variables as explanatory factors. Overall, we checked that all model assumptions were met (e.g., homoscedasticity and normality of residuals) using the *DHARMa* package. To this end, we log-transformed male locomotor performance and log+1-transformed the aggregation scores. In the

model where we found a significant interaction, we conducted pairwise comparisons using the estimated marginal means of the models and we corrected P values for multiple testing using the Tukey method, as implemented in the *emmeans* R package. Finally, we conducted power analyses for each of our statistical models using the packages *pscl* (Jackman et al., 2024), *pwr* (Champely et al., 2020) and *powerSurvEpi* (Qiu et al., 2021).

RESULTS

Overall, we detected no main association between forceps length and the six traits measured (Table 1). This applied to male locomotor performance, likelihood of fleeing after a physical disturbance, aggregation score, sperm storage and survival after pathogen infection (Figure 3; Table 1). There was also no difference between males with short and long forceps in terms of survival under harsh environmental conditions, although males with long forceps survived better when they came from Cinais compared to Valence (this trend was not present in males with short forceps; Figure 3; interaction in Table 1; pairwise comparisons: Long forceps Cinais vs Valence: Z = -3.311; P = 0.005; Short forceps Cinais vs Valence: Z = -0.611; P = 0.929). Regardless of forceps length, males from Cinais were less likely to flee after a physical disturbance and less gregarious than males from Valence (Table 1). These differences between the two populations were absent for all other traits measured (Table 1). There was no interaction between the population of origin and forceps length for any of the traits measured, except for survival under harsh environmental conditions (Table 1). The statistical power of each analysis is shown in Table 1.

DISCUSSION

The display of large, extravagant weapons often comes at a cost to the males (Emlen, 2008; Goldberg et al., 2019; McCullough et al., 2016). It is therefore expected that only males in the best condition can afford them and thus that these large weapons signal good male quality (Otte & Stayman, 1979; Emlen & Nijhout, 2000). Here, we tested this prediction in the European earwig by investigating whether male forceps length is associated with six important behavioural and physiological traits. Contrary to predictions, our experiment did not allow us to detect an association between forceps length and locomotor performance, boldness (i.e., likelihood to flee after a physical disturbance), aggregation behaviour, sperm production, and male survival after pathogen infection. These findings were consistent between the two populations, although some of the traits measured were population specific: males from Cinais were generally bolder, less gregarious and (only if they carried long-forceps) had a better chance of surviving in harsh conditions than males from Valence.

Our results contrast with much of the literature reporting associations between sexually selected male attributes and physiological, behavioural or immunological traits in arthropods (Emlen, 2008; Goldberg et al., 2019; McCullough et al., 2016). However, the European earwig is not the only case where such associations appear to be lacking (Emlen, 2008; Swallow & Husak, 2011). For example, carrying giant claw is not associated with the efficiency of escape behavior and the level of metabolic costs in two fiddler crabs (Pena & Levinton, 2021; Tullis & Straube, 2017). Similarly, bearing large horns does not reflect male growth, mobility, or immunity in the rhinoceros beetle *Trypoxylus dichotomus* (McCullough & Emlen, 2013; McCullough & Tobalske, 2013). It has been suggested that this apparent lack of general association may be due to the fact

294 that the performance of each weapon size depend on the environment in which the weapons are
295 used and/or because weapon sizes reflect alternative reproductive tactics (McCullough et al.,
296 2016; McCullough & Emlen, 2013). This could be the case with the European earwig (Tomkins et
297 al., 2005). In this species, males with large forceps have been suggested to be more active in
298 guarding of females, while males with small forceps are more active in sneaking into copulations
299 (Kamimura, 2014; Tomkins & Brown, 2004). Having long forceps should therefore be rewarded
300 more frequently when the encounter rate between competitors is high (Hunt & Simmons, 2001),
301 such as in high population densities. Consistent with this prediction, Tomkins & Brown (2004)
302 found that the proportion of long forceps males increased with population densities across 46
303 island and mainland sites in the UK. Another possible explanation for our results is that the
304 association between male forceps length and the six traits measured could have been masked by
305 differences in the amount of resources available to each male and/or used by each male for these
306 traits (van Noordwijk & de Jong, 1986). In our study, all males were kept under identical laboratory
307 conditions and fed *ad libitum* for three months prior to our measurement. This explanation would
308 therefore suggest that the variation in resources influencing investment decisions is determined
309 prior to field sampling, e.g. during development or early adulthood. Previous data suggest that it
310 could be the case in the European earwig. In this species, early life conditions have long-term
311 effects on the physiology and behaviour of adults (Raveh et al., 2016; Thesing et al., 2015; Wong
312 & Kölliker, 2014) and inter-individual variation in female condition can mask investment trade-off
313 between egg quantity and quality (Koch & Meunier, 2014). Overall, our findings call for future
314 studies to confirm whether males with short or long forceps have alternative reproductive tactics,
315 whether their reproductive success depends on population densities and whether early life

316 conditions can affect the likelihood to detect the association between forceps length and the six
317 traits measured. They also call for further research to quantify other potential costs of carrying
318 long forceps in this species, for example in terms of predation rates and ability to fly (Crumb &
319 Eide, 1941). These notwithstanding, our findings emphasize that forceps size is not a good
320 predictor of the six behavioural and physiological traits measured in males.

321 While we found no overall difference between short- and long-forceps males, our data
322 reveal population differences in terms of males' boldness, aggregation level and resistance to
323 starvation. Cinais had males that were generally bolder, less gregarious, as well as males with long
324 forceps that survived food deprivation better than Valence. These effects are unlikely to reflect a
325 plastic response to the direct environment of the males tested (e.g. population differences in
326 terms of nutritional status), as they were all reared under common laboratory conditions for
327 months prior to the start of our experiments. Instead, it could reflect population idiosyncrasies
328 that have affected their development, such as climatic conditions (Valence is warmer than Cinais),
329 environmental conditions and/or exposure to phytosanitary products (e.g. Valence is a cultivated
330 orchard, whereas Cinais is an uncultivated forest edge), or population-specific genetic background.
331 In line with this hypothesis, several life history traits of the European earwig are shaped by the
332 duration of cold during egg development, the level of warm temperatures during nymph
333 development and, more generally, by seasonal parameters encountered by offspring during
334 development (Coulm & Meunier, 2021; Körner et al., 2018; Tourneur & Meunier, 2020). Similarly,
335 recent studies have shown that the European earwig can be sensitive to exposure to even low
336 levels of pesticides, which can lead to populations specificities in terms of earwig physiology,
337 morphology and behaviour (Fricaux et al., 2023; Le Navenant et al., 2019; Malagnoux, Capowiez,

et al., 2015; Malagnoux, Marliac, et al., 2015; Mauduit et al., 2021; Meunier et al., 2020). Regardless of the nature of these population idiosyncrasies, our study shows that they do not affect the (lack of) association between forceps length and the six traits measured.

Overall, our findings questions the robustness of our understanding of forceps length diversity in terms of function, maintenance and use as a possible quality signal in the European earwig (McCullough et al., 2016). To date, our knowledge of these questions is exclusively based on laboratory experiments in which females were presented to one or two males (reviewed in Kamimura, 2014). While the results of these studies suggest that males can gain fitness benefit from growing longer forceps (Forslund, 2000, 2003; Radesäter & Halldórsdóttir, 1993; Styrsky & Rhein, 1999; Walker & Fell, 2001), they contain two important limitations. First, they considered mating success (i.e., success in gaining copulation) and not reproductive success (i.e. success in producing offspring). As these two parameters are not necessarily related (Thompson et al., 2011), it cannot be excluded that short forceps males have a similar or even higher reproductive output than their counterparts. This could be because mating is more efficient in short compared to long forceps males (Brown, 2006), or because short forceps males have alternative reproductive tactics to long forceps males (see above; Tomkins & Brown, 2004). One study used a genetic approach to study reproductive success and found no association between male forceps length and the number of offspring sired (Sandrin et al., 2015). However, this study only focused on length variation within short forceps males. The second limitation is that all these studies have examined mating success in groups of a maximum of two males. This number is far less than the hundreds of individuals that typically constitute earwig aggregates (Meunier, 2024) and thus cannot rule out the possibility that the apparent advantage of long-forceps males in pairs or trios may disappear

or even become a disadvantage under different demographic conditions (Hunt & Simmons, 2001; Oliveira et al., 2008; Tomkins & Brown, 2004). Hence, our current understanding of the relationship between forceps length and male fitness should be treated with caution in the European earwig. Our results thus call for future studies to determine the function and reliability of male forceps length under natural conditions, and the evolutionary drivers that explain the maintenance of its diversity within and between populations.

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Data, script and code availability

All data and R script are available online: https://doi.org/10.5281/zenodo.11469628

Conflict of interest disclosure

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592

593 **Table 1** – Effects of male forceps length and population of origin of the three physiological and

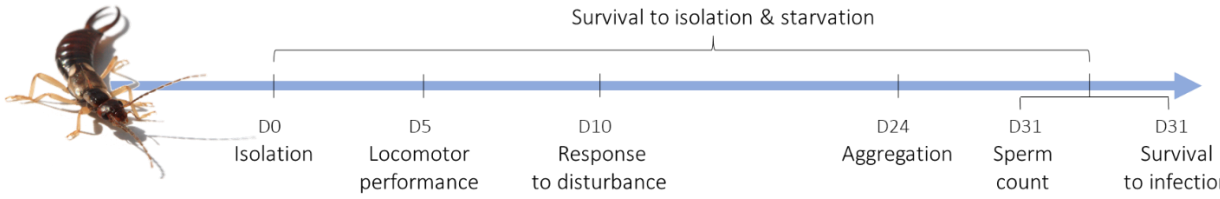
594 three behaviours measured in this study. Significant p-values are in bold.

595

	Population of origin	Forceps length	Interaction	Statistical power
Locomotor performance	$F_{1,115} = 0.21$ P = 0.648	$F_{1,115} = 1.14$ P = 0.287	$F_{1,115} = 1.25$ P = 0.265	0.241
Likelihood of fleeing away after a physical disturbance	LR $\chi^2_1 = 8.74$ P = **0.003**	LR $\chi^2_1 = 1.06$ P = 0.303	LR $\chi^2_1 = 0.57$ P = 0.450	0.239
Aggregation score	$F_{1,88} = 3.83$ P = **0.054**	$F_{1,88} = 0.004$ P = 0.948	$F_{1,88} = 0.02$ P = 0.878	0.347
Survival rate under harsh environmental conditions	LR $\chi^2_1 = 7.88$ P = **0.005**	LR $\chi^2_1 = 0.02$ P = 0.885	LR $\chi^2_1 = 4.22$ P = **0.039**	0.447
Sperm count	$F_{1,35} = 0.42$ P = 0.522	$F_{1,35} = 0.19$ P = 0.667	$F_{1,35} = 0.75$ P = 0.391	0.155
Survival rate after infection	LR $\chi^2_1 = 0.02$ P = 0.891	LR $\chi^2_1 = 0.15$ P = 0.695	LR $\chi^2_1 = 0.46$ P = 0.496	0.432

596

Figure 1 – Timeline of the experimental design and evolution of sample size over the course of the experiment. The observed decrease in sample size over time reflects their mortality during isolation and in the absence of any food source. Males are distributed according to their forceps length category and population of origin.

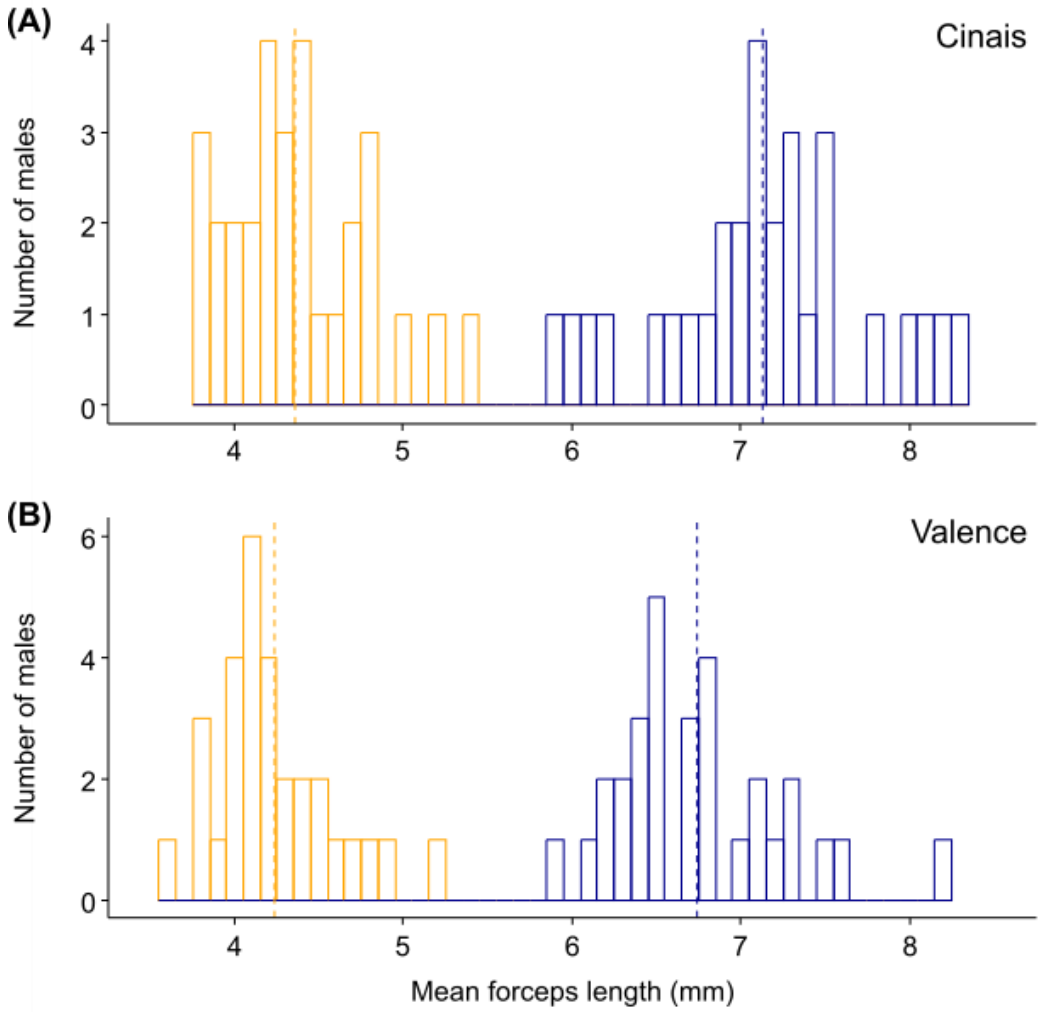


Long forceps	Cinais	N=30	30	30	18	9	8
	Valence	N=30	29	29	28	10	18
Short forceps	Cinais	N=30	30	30	22	10	12
	Valence	N=30	30	30	24	10	14

602 **Figure 2** – Forceps length distribution of the 120 selected males with (blue) short and (orange)

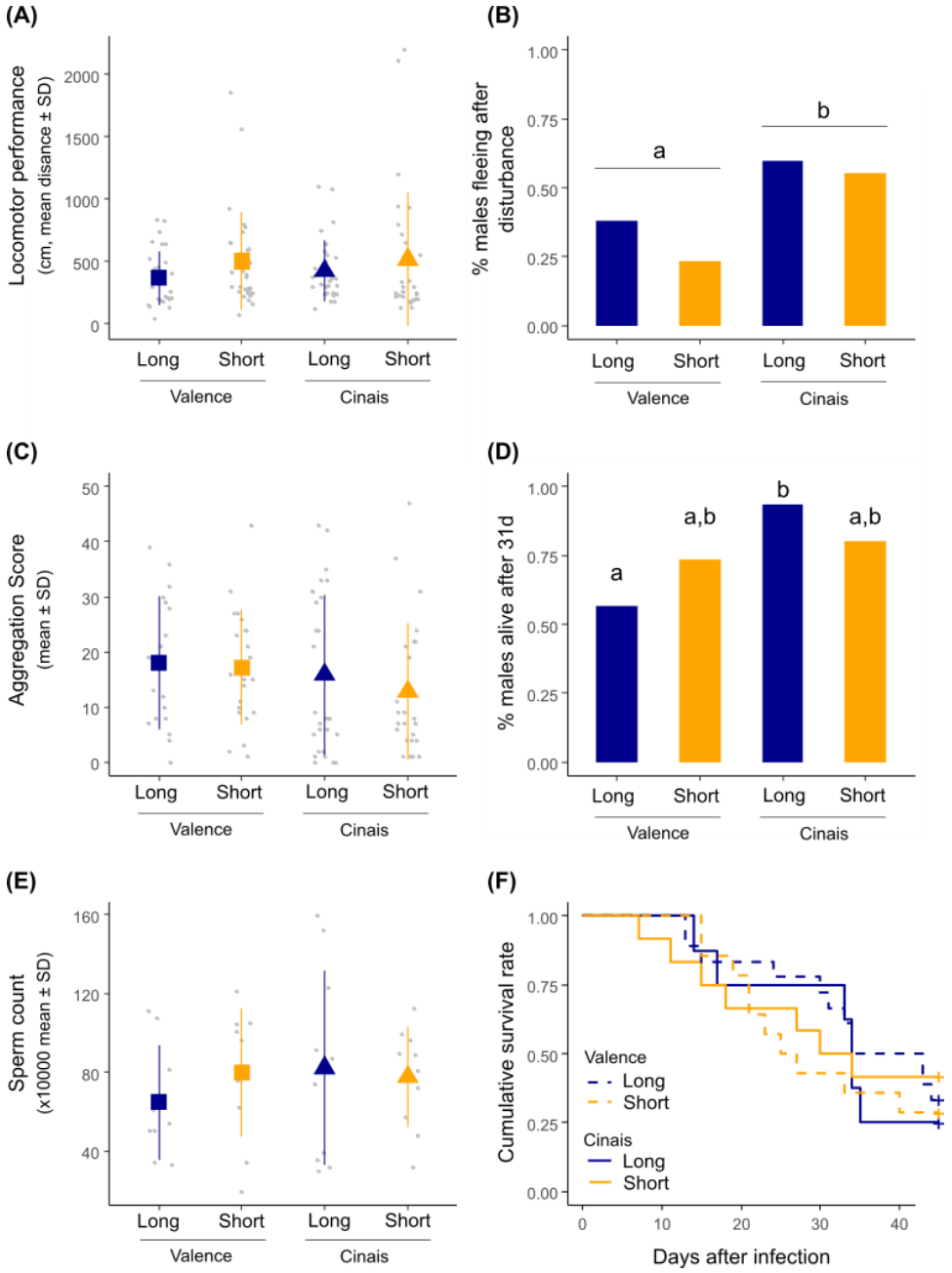
603 long forceps in (A) Cinais and (B) Valence populations. Dashed lines show the mean values per

604 forceps category for each population.



605

606 **Figure 3** – Association between forceps length and male (A) locomotor performance, (B) likelihood
607 of fleeing after a physical disturbance, (C) propensity to aggregate with conspecifics, (D) likelihood
608 of being alive after 31 days in social isolation and without food access, (E) sperm storage in the
609 vesicle and (F) survival rate after pathogen infection. Males came from Cinais (square) or Valence
610 (triangle). Different letters P <0.05.



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