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Blandine Charrat, Dominique Allainé, Jean-François Lemaître, Nelly Burlet, Camille Mermet-Bouvier, et al.. Social interactions throughout life impair longevity and age-specific mating success in male parasitoids. *Behavioral Ecology and Sociobiology*, 2024, 78, pp.90. 10.1007/s00265-024-03506-y . hal-04668711

HAL Id: hal-04668711

<https://hal.science/hal-04668711v1>

Submitted on 7 Aug 2024

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Social interactions throughout life impair longevity and age-specific mating success in male parasitoids

Authors:

Blandine Charrat*¹ (ORCID: 0000-0002-3730-0387), Dominique Allainé¹ (ORCID: 0000-0001-9767-5992), Jean-François Lemaître¹ (ORCID: 0000-0001-9898-2353), Nelly Burlet¹ (ORCID: 0000-0003-3231-4154), Camille Mermet-Bouvier¹ (ORCID: 0000-0002-9192-5046), Christophe Bressac² (ORCID: 0000-0001-7609-8970), Isabelle Amat¹ (ORCID: 0000-0003-1058-9309) & Emmanuel Desouhant¹ (ORCID: 0000-0003-0317-4463)

¹: Université Claude Bernard Lyon 1, CNRS, VetAgro Sup, UMR 5558 Biométrie et Biologie Evolutive, Bat. G Mendel 43 bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France

²: Institut de Recherche sur la Biologie de l’Insecte (IRBI), UMR 7261 CNRS-University of Tours, 37200 Tours, France

*: corresponding authors: blandine.charrat@gmail.com

Abstract:

Social interactions among individuals within a species profoundly influence behavioural and life history traits, impacting fitness. While extensively studied in cooperative and eusocial species, the effects of social environment on fitness in non-social species, particularly insects, remain less explored. Our study investigates the impact of social environment, specifically male density, on fitness-related traits in the hymenopteran parasitoid *Venturia canescens*. The research focuses on longevity, reproductive behaviours (latency before mating, mating probability and duration), and offspring production capacity. Through a lifespan study, males were exposed to either isolation or

regular encounters with conspecific males, alternating with periods of female presence or absence. Results show a trend of reduced longevity in socially exposed males and a significant decline in mating success with age in the social context. However, reproductive behaviours and offspring produced by males remain unaffected. This study sheds light on the intricate interplay between social environment, ageing, and reproductive strategies in non-social insect species, emphasizing the need for further exploration of social effects on male behaviour and notably potential influences of interactions between male and females but also between females.

Keywords: *Venturia canescens*, senescence, reproductive experience, physical interactions, past social information

Statements and Declarations

Significance Statement: Impacts of social interactions on individuals were largely explored in social species, but remain little studied in non-social ones. This study aims at testing the impact of male-male interactions before the encounter of a female (past social interactions) on male reproductive behaviours and longevity in a non-social parasitoid wasp. We show that longevity is negatively affected by the past social interactions in this wasp. Those interactions also impose a decline in male mating probability with age (senescence pattern) or mating experience (number of matings). Latency before mating, mating duration and offspring production are not influenced by social environment. On the other hand, offspring production increases with mating experience.

Conflict of interest: The authors declare to have no conflict of interest.

Contribution Statement: All authors contributed to the study conception and design. Experimentations by B. Charrat, N. Burllet and C. Mermet-Bouvier and analyses were performed by B. Charrat. Interpretations of the results and the first draft of the manuscript was done by B. Charrat, I. Amat, D. Allainé and E. Desouhant and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability Statement: The datasets generated during and/or analysed during the current study are available in the figshare repository, <https://figshare.com/s/f7563a28ce76c605fb8b> (permanent link (DOI) will be given after acceptance).

1 Introduction

2 Within species, social interactions take multiple forms from grooming behaviour, exchange of
3 information, competitive interferences, or helping during the parental care or foraging period
4 (Tinbergen 1990; Giraldeau and Caraco 2000; Wong et al. 2013; Braga Goncalves et al. 2022). At the
5 individual level, this social environment, represented by the interactions between the focal individual
6 and the group of individuals surrounding, is acknowledged to impact a wide spectrum of behavioural
7 and life history traits, and to ultimately modulate its fitness (reviewed in Van Den Bos et al. 2013).
8 The social environment may result in social interactions occurring before or between breeding
9 episodes for iteroparous species (hereafter called past information) or interactions that take place
10 during breeding opportunities (present information). Empirical studies performed in a wide range of
11 species have provided compelling evidence for the profound influence of social environment
12 experienced throughout life on fitness-related traits in both males and females, especially longevity
13 and reproductive success (e.g. in birds Brouwer et al. 2014; and in primates Thompson 2019). For
14 instance, female baboons (*Papio hamadryas ursinus*) who form tight social bonds with conspecific
15 females display a longer longevity compared to females showing much fewer social skills (Silk et al.
16 2010). More recently, studies investigating the fitness costs of adverse social environments have
17 started to focus on ageing (Lucas and Keller 2020). They evidence that poor social environment
18 strengthens the intensity of both actuarial senescence (i.e. increase in mortality risk with increasing
19 age) and reproductive senescence (i.e. decrease in reproductive performance with increasing age)
20 (Korb and Heinze 2021). Yet these studies remain largely limited to cooperative breeders (Berger et
21 al. 2018) or eusocial species (e.g. Tasaki et al. 2021), which calls for studies investigating this topic in
22 non-social species. Overall, these social environment effects are now known to be mediated by various
23 and intertwined genetic and physiological processes such as gene expression profiles (Ben-Shahar
24 2002), immune performance (Snyder-Mackler et al. 2016) or telomere dynamics (Hammers et al.
25 2019).

26 For a long time, non-social animals have not been the focus of studies testing how social
27 environments influence fitness. Nevertheless, social interactions among males in non-social insects at

28 different stages of their life can impact their reproductive behaviours and opportunities of reproduction
29 during their lifetime (for a review: Prokopy and Roitberg 2001). In many non-social insect species,
30 groups of interacting males may form near the females during a mating event. The intra-sexual
31 competition that ensues may have detrimental consequences for male reproductive success. The
32 density of rival males may also alter male reproductive behaviours, such as reproductive allocation by
33 changing the value that the contestants allocate to the females (Briffa 2008). Males of the golden egg
34 bug, *Phyllomorpha laciniata*, increase the copulation duration and the rate of sperm transfer in
35 presence of rivals (Garcia-Gonzalez and Gomendio 2004). This pattern is also documented in walnut
36 flies *Rhagoletis juglandis* (Alonso-Pimentel and Papaj 1996). The social environment experienced
37 before the occurrence of mating also influences subsequent reproductive success and survival
38 prospects in non-social species particularly in *Drosophila* species. In *D. melanogaster*, males exposed
39 to rivals throughout life (i.e. during and between breeding opportunities) incur costs in response to the
40 potential sexual competition: males in presence of conspecific males have fewer mating opportunities
41 in later life and had shorter life spans than controls (Bretman et al. 2013). Thus, both precopulatory
42 and postcopulatory traits are impacted by the social environment experienced by males. For example,
43 perception of future risk of sperm competition (i.e. the probability that sperm from a given male will
44 be in competition for fertilization with sperm from another male, Parker et al. 1997) in *D.*
45 *pseudoobscura* and *D. subobscura*, leads respectively to an increase of ejaculate size and increases the
46 cost of reproduction in terms of longevity (Price et al. 2012; Bretman et al. 2013; Lizé et al. 2014). On
47 the other hand, male bruchids *Callosobruchus maculatus* provide an example of such rapid and plastic
48 response to social context with positive effect. When reared in isolation, seeing a conspecific even for
49 a short period (10 minutes) increases males' longevity (Amiri and Bandani 2022), and therefore
50 potentially the number of mating opportunities. Present and past effects of social information are not
51 mutually exclusive and both influence behaviours. In *Drosophila* species, both lead to modifications
52 of male's courtship behaviours (for a review Chen and Sokolowski 2022). However, they can also
53 differentially influence latency before mating and mating duration in males and females, potentially
54 leading to intersexual conflicts (Fowler et al. 2022). Thus, the repetition of social experiences over the

55 lifespan of insects, whether social (Amdam 2011) or not (Leech et al. 2017), impact both actuarial and
56 reproductive senescence patterns (Rodríguez-Muñoz et al. 2019; Quigley and Amdam 2021).

57 The hymenopteran parasitoid *Venturia canescens* (Ichneumonidae) is a relevant biological
58 model to quantify the effects of social environment on reproductive behaviours throughout the life
59 course. It was already demonstrated that interactions between females exploiting a host patch provide
60 information used by other foraging females to avoid this patch and the associated risk of intraspecific
61 competition for oviposition (Castelo et al. 2003). However, the impact of the social environment on
62 males has not yet been investigated. Several characteristics indicate that males are confronted with
63 other conspecifics in varying densities during their lives, and that these social interactions could have
64 direct and indirect effects on their behaviours. For example, these polygynous males may be
65 confronted several times during their lifetime (average lifespan under laboratory conditions = 16 days;
66 Charrat et al. 2023) with groups of males at egg-laying sites where males and females meet ("rendez-
67 vous" sites, observed in the laboratory (Metzger et al. 2010) and in the field (E.D. pers. obs.)). We
68 assume that the males may acquire and use the information on their social environment to adjust their
69 future reproductive tactics by modulating their sexual behaviours.

70 Thus, in this study, our aim was to test the effect of the past social environment on male
71 fitness-related traits, focussing on one aspect of the social environment, namely male density. We
72 tested in a single experiment the effects of past social environment on three main traits: i) longevity, ii)
73 reproductive behaviours (latency before mating, mating probability and mating duration), and iii)
74 offspring production capacity (sex-ratio). More specifically, we tested the effect of past social
75 information throughout life course by placing focal males in the presence of constant density of
76 conspecifics males at regular intervals, alternating with periods when these focal males accessed
77 female without the male conspecifics. Tracking males throughout their lifetime enabled us to describe
78 the age-specific dynamics of reproductive behaviours and offspring production, and thus the
79 reproductive senescence. We predicted that interactions between males incur physiological costs that
80 increase their reproductive senescence (e.g. age-specific decline in mating probability), reduce their
81 longevity and subsequent lifetime reproductive success in comparison to males living in isolation.

82

83 **Materials and Methods**

84 **Insect and rearing facilities**

85 Experiments were conducted using a sexual (parthenogenic arrhenotokous) strain of the parasitoid
86 *Venturia canescens* (Hymenopteran, ichneumonidae). This strain was established from large number
87 of individuals caught in several sampling dates in an organic orchard near Valence, France (GPS:
88 44.93°N; 4.90°E, Gotheron station, INRAE) during summer 2020. Female *V. canescens* parasitize a
89 wide range of moth species (Salt 1976). This parasitoid species is solitary and koinobiont, where each
90 parasitized host allows the emergence of at most one adult wasp, and continues to develop after
91 parasitization. The wasps were reared under laboratory conditions ($24\pm 1^{\circ}\text{C}$, $50\%\pm 10\%$ relative
92 humidity, DL 12:12). The pyralid larvae of *Ephestia kuehniella* fed with semolina were used as hosts.
93 All experiments were conducted under the same laboratory conditions as rearing.

94 **Reproductive biology of *V. canescens***

95 Since the females are monandrous (Collet et al. 2020) one expects that male competition results in
96 reduction of latency before mating allowing the fastest male to access female (Charrat et al. 2023).
97 Mating duration might be sensitive to rival males in particular conditions: i) if the mating duration is
98 positively correlated with the number of sperm transferred (and thus the capacity to fertilize eggs and
99 produce daughters), ii) if the number of potential mating for a male is reduced (which is the case in
100 natural populations of *V. canescens*, (Collet et al. 2020). The haplodiploid sex determination in
101 Hymenoptera allows us to evaluate the offspring production capacity of males by calculating the
102 offspring sex-ratio produced for a given mating, as male's progeny is only composed of daughters.
103 The sex-ratio being the number of sons divided by the total number of offspring, this measure is
104 inversely proportional to the reproduction capacity of a given male. The number of daughters per male
105 was not used because of the huge variability in the number of offspring produced among females
106 under non-limiting host availability conditions.

107 **Effects of social environment on male longevity and reproductive senescence**

108 We conducted a single experiment with two main goals. First, we quantified the effect of social
109 environment, defined as the density of males encountered outside the reproductive period (i.e. when

110 males did not encounter females, see below) on male longevity. Second, we investigated the effect of
111 social environment on reproductive senescence by testing i) whether the age-specific allocation to
112 reproductive behaviours (latency before mating, mating duration and mating probability) throughout
113 their life are influenced by their social environment; ii) how the social environment alters the age-
114 specific dynamics of offspring production by males. Because age and mating experience
115 (characterized by mating numbers) may be correlated we also assessed the effect of this mating
116 experience. A global diagram of the experimental protocol is presented in Fig. 6 (Appendix 1).

117 To fulfil these objectives, we compared males either kept in isolation (no interaction with counterparts,
118 hereafter referred to as *Isolated*) or regularly brought into the presence of male conspecifics (hereafter
119 referred to as *Social*). Both categories of males had periodically access to females without any other
120 conspecific (called “female presence”) and these periods were interspersed with isolation periods or
121 encounters with males and called “female absence”.

122 The experimental procedure was as follows. We isolated males at emergence (day 0) and kept them
123 individually in plastic tubes (diameter 3cm x height 7cm). They were randomly assigned to one of two
124 treatments of social environment (*Isolated* or *Social*). These males were defined as focal males since
125 we recorded their behaviours and life history traits until death (see below). To differentiate focal males
126 from the others, all focal males (either from *Social* or *Isolated* condition) were marked by a dot of
127 white water-paint on the thorax; the paint inducing no behavioural changes (Fauvergue et al. 2015).

128 During “female presence” periods (i.e. days 1, 4, 6, 8, 11 and 13), each focal male had an opportunity
129 to mate. Males were individually placed in a new plastic tube with a 1-day-old virgin female, without
130 food, during 3 hours as preliminary analyses revealed that this duration allowed a large proportion
131 (around 70%) of individuals to mate ($n = 20$, unpublished data). Before being used for mating, the
132 females were stored individually in plastic tube with honey. Food was removed from the males and
133 females 2 hours before observation started, in order to standardize the feeding status of individuals.
134 For each female presence period, duration of copulation and latency before mating were recorded
135 visually with an event recorder (BORIS, Friard and Gamba 2016). Up to 12 pairs were observed at the
136 same time. At the end of the observation time, the focal males were replaced in their individual plastic
137 tube. In order to quantify the offspring production capacity of males, we used sex-ratio calculated

138 among offspring from each mating. To do so the mated females were individually placed in a box
139 containing hosts *ad libitum* and food. We calculated the sex-ratio among the offspring emerged up to 5
140 weeks after the day the female was placed in the box. Studying the sex-ratio over this entire period
141 enabled us to free ourselves from any potential bias in the production of sons and daughters, the sex-
142 ratio being slightly biased towards females on the first day after mating (Metzger et al. 2008).
143 Moreover, as females of this species do not regulate their offspring sex-ratio (Metzger 2008; Metzger
144 et al. 2008), this latter can be considered as a proxy of the male's offspring production capacity. Mean
145 offspring sex-ratio was negatively correlated to the mean number of daughters produced by each male
146 in the two social conditions (*Isolated*: $r = -0.68$, $p = 0.004$ (large effect size); *Social*: $r = -0.42$, $p = 0.08$
147 (medium to large effect size), which indicates that male with, in average, more female-biased sex-
148 ratios produced in average more daughters. Therefore, sex-ratio is a good proxy of offspring
149 production capacity.

150 During the "female absence" periods (i.e. days 2, 3, 5, 7, 9, 10 and 12), each focal male from *Social*
151 treatment was placed in a plastic tube with 3 other males (aged between 1 and 7 days old), randomly
152 selected from the pool of non-focal males, for 8 hours (from 9am to 5pm), with food (honey) *ad*
153 *libitum*. Just after, the focal males were replaced individually in their plastic tube. In the meantime, the
154 focal males from *Isolated* treatment were kept in their individual tubes, without any other males. The
155 non-focal males were stored in a population cage, with food (honey) *ad libitum*.

156 We quantified the longevity of focal males by checking twice a day (at 8am and 6pm) their survival
157 status. Longevity was estimated by the median between the time found dead and the last time the
158 individual was observed alive. Once dead, focal males were stored in a freezer for size measurements.
159 The size of individuals was estimated by measuring the hind left tibia length (Pelosse et al. 2011),
160 using a dissecting microscope with a camera (AxioCam, software Zen core v3.0). A total of 24 focal
161 males per treatment of social environment (*Isolated*, *Social*) were tested.

162 **Data analysis**

163 For all experiments, male size was initially added in statistical models as covariable, as there is
164 evidence that in *V. canescens* the size is positively associated with lifespan and reproductive success
165 (Pelosse et al. 2011; Amat et al. 2017). Its effect being always non-significant, we removed it

166 thereafter. All generalized linear mixed models and mixed linear models included male identity as
167 random factor. We presented the results from the simplest model selected after backward procedure
168 (Crawley 2012) except for the model selection procedure concerning reproductive senescence
169 (described hereafter). All analyses were performed with R (R Core Team 2020).

170 *Effect of social environment on male longevity*

171 We tested the global effect of the social environment (*Isolated* vs. *Social*) on male longevity by the
172 mean of a Kaplan-Meier analysis and a log-rank test. We then tested the effect of social interactions,
173 mating experience (i.e. number of matings carried out) and their interaction on male longevity using a
174 cox model, with two time-dependent variables: “Social environment”, accounting for the number of
175 times the focal male had encountered groups of 3 counterparts and “Mating”, the number of matings
176 the focal male had already performed. The Cox model allows to evaluate the effect of each encounter
177 and each mating on risk of death. The Proportional Hazards Assumption was verified (the test of the
178 relationship between scaled Schoenfeld residuals and time was non-significant for each variable,
179 mating: $\chi^2 = 2.55$, $df = 1$, $p = 0.11$, social environment: $\chi^2 = 0.7$, $df = 1$, $p = 0.40$ and for the global
180 test: $\chi^2 = 4.61$, $df = 2$, $p = 0.10$).

181 *Effect of social environment and male age or mating experience on reproductive behaviour and* 182 *offspring production capacity*

183 To investigate whether the reproductive behaviour and offspring production capacity of males differ
184 between the two social environments, we fitted models with either ‘age’ or ‘mating experience’
185 (defined as the number of matings previously performed) as explanatory quantitative variables. We did
186 not include male age and male number of matings in the same model because these two variables were
187 correlated ($r = 0.83$) and could lead to collinearity issues (Dormann et al. 2013). In order to compare
188 the fit of the two statistical models with age or number of matings as explanatory variable, we
189 calculated QIC for GEE models (Pan 2001). QIC is interpreted in the same way as AICc for GLMM
190 and LMM, with the lowest QIC corresponding to the best fit. In the following subsections, we describe
191 more precisely the fitted model for each response variable tested.

192

193 *i) Effect of social environment on male mating success*

194 We first analysed the effect of social environment (*Isolated* and *Social*), age or mating experience and
195 their interaction on male's mating success (0 when the male did not mate, and 1 otherwise) by fitting a
196 generalized estimating equation (GEE) (*geepack* package, `geeglm()` function, Halekoh et al. 2006). As
197 a measure of mating experience, we used the probability of mating instead of the total number of
198 matings per age because the latter depends greatly on the longevity of the males, therefore the number
199 of males (and of potential matings) is not constant through the experiment. The GEE was fitted with a
200 binomial distribution, unstructured correlation matrix and clustered by the identity of the males. This
201 clustering allows to take into account the potential correlations between measures of the same male
202 (Halekoh et al. 2006). GEE was used for this analysis because GLMM failed to converge due to
203 limited number of repetitions per male.

204 *ii) Effect of social environment on male reproductive behaviour*

205 In this section, the analyses with male age and mating experience as explanatory variables differed.
206 First, we tested different senescence patterns in reproductive behaviour according to age fitting mixed
207 linear models (LMM) with male identity as random factor. Thus, we tested the effect of male age,
208 social environment (*Isolated* and *Social*) and their interaction on two behavioural traits (i.e. latency
209 before mating and copulation duration). To describe senescence, we considered possible non-linear
210 effects of age (as generally observed in reproductive senescence studies, e.g. Cambreling et al. 2023).
211 We thus compared models with a linear effect of age, a quadratic effect of age, a threshold effect of
212 age (with three possible different patterns: constant then slope, slope then constant or two slopes) and
213 a model without age effect (i.e. with only male size as a covariate). The full list of fitted models is
214 provided in Appendix 2 for mating latency and Appendix 3 for copulation duration. We selected the
215 model with the smaller AICc (Burnham et al. 2002). When the difference in AICc between two
216 competing models was less than 2, we selected the model with the lower number of parameters. For
217 the latency before mating, the best model was the model with a threshold effect of age (threshold = 11
218 days) and two slopes. For the copulation duration, the best model was the null one.

219 Second, we tested the effect of male mating experience, social environment and their interaction on
220 the same two behavioural traits (latency to mate and copulation duration) by fitting GEE with Gamma
221 distribution and unstructured correlation matrix and clustered by the identity of the males. GEE were

222 used for this analysis because GLMM failed to converge due to limited number of repetitions per
223 male.

224 *iii) Effect of social environment on offspring production capacity*

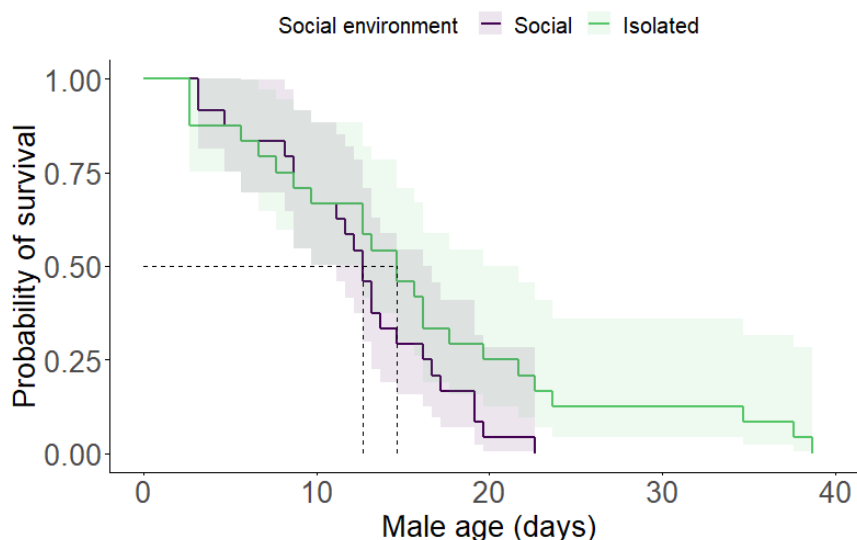
225 We fitted mixed generalized models (GLMM) with a binomial distribution to test the effect of social
226 environment in interaction with male age or mating experience on sex-ratio for each mating of males.
227 Male identity was included as a random factor.

228

229 **Results**

230 *Effect of social environment on male longevity*

231 We detected a trend for an effect of the social environment on male longevity (Fig. 1). Males in the
232 social treatment group (*Social*) had a shorter lifespan compared to males in isolated condition
233 (*Isolated*) ($\chi^2 = 2.9$, $df = 1$, $p = 0.09$, small to medium effect size Glass' delta = -0.33, IC = [-0.79,
234 0.13]). The median longevity (i.e. age at which 50% of the pool of individuals is dead) was 12.7 and
235 14.7 days for individuals in the *Social* and *Isolated* groups, respectively. When considering
236 survivorship to 20% (i.e. age at which 80% of the pool of individuals is dead), the difference increased
237 with a longevity of 16.9 and 22.1 days for individuals in the *Social* and *Isolated* groups, respectively.
238 Each encounter with other males increased male's death probability ($e^{\beta} = 1.15$, $\chi^2 = 5.56$, $df = 1$, $p =$
239 0.018). However, the number of matings for a given male did not impact its risk of dying ($e^{\beta} = 1.13$, χ^2
240 $= 0.13$, $df = 1$, $p = 0.72$).



241

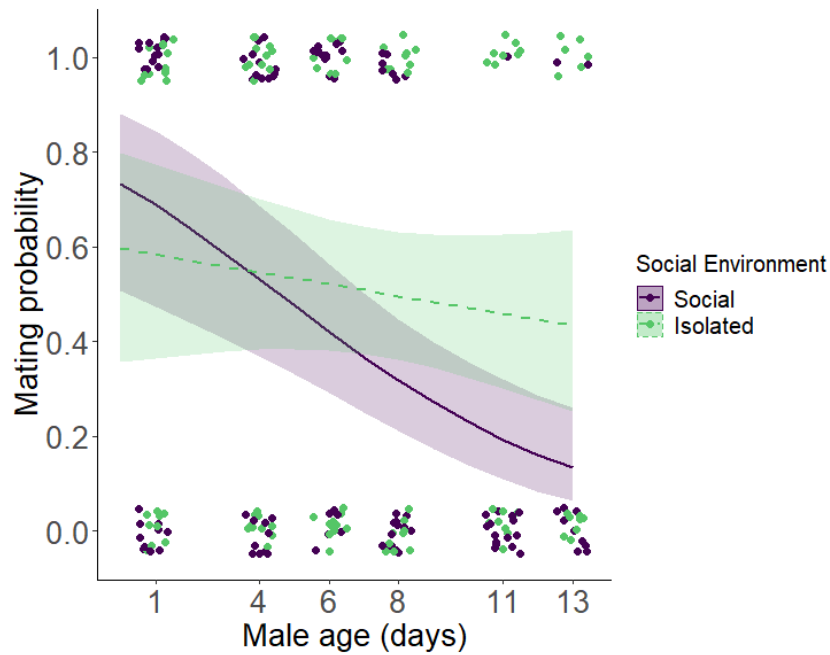
242

243 **Fig. 1** Survival probability of males according to age and condition of social environment. Data are
 244 represented through Kaplan-Meier representation and confidence intervals (coloured areas). Dotted
 245 lines represent the survivorship 50% for both treatments of social environment (*Social* versus *Isolated*
 246 conditions)

247 *Effect of social environment and male age or mating experience on reproductive behaviour and*
 248 *offspring production capacity*

249 *i) Effect of social environment on male mating success*

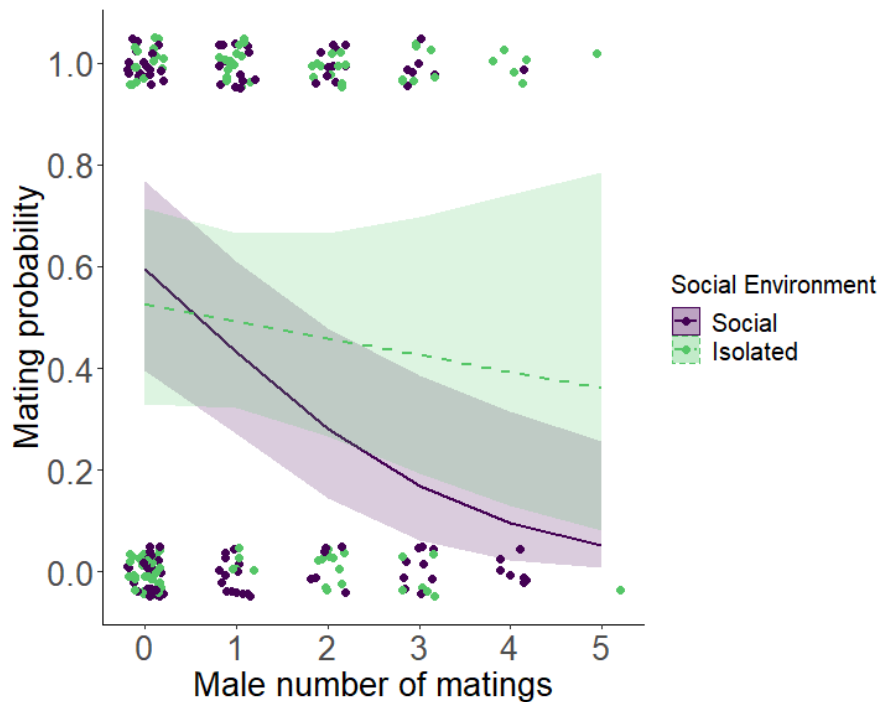
250 The social environment influenced the pattern of senescence in males mating success (estimated by the
 251 mating probability, see Fig. 2), as evidence by the significant interaction between male age and social
 252 environment (GEE: $\chi^2 = 6.64$, $df = 1$, $p = 0.001$). Mating success decreased strongly with age in males
 253 from the *Social* groups (9.04 times fewer males mated on day 11 than on day 1, among males alive
 254 respectively on days 1 and 11). On the other hand, the mating probability remained nearly constant in
 255 *Isolated* males (1.14 times fewer males mated on day 11 than on day 1).



256

257 **Fig. 2** Age-specific male mating success (estimated by mating probability, see methods) according
 258 social environment (*Isolated* and *Social* condition). We represented model predictions and their
 259 confidence interval (coloured areas). Mating success = 0 indicates that male did not mate; mating
 260 success = 1 indicates that males mated. Dots represent mating observations in different ages. In order
 261 to avoid overlapping dots, they have been spaced out in the figure

262 The same trend is observed when considering male mating experience. Indeed, the effect of the mating
 263 experience (i.e. number of matings performed for each male) on mating success depends on the social
 264 environment (interaction: $\chi^2 = 3.87$, $df = 1$, $p = 0.049$). Mating probability decreased with the number
 265 of previous mating events in males from *Social* condition but remained nearly constant in *Isolated*
 266 males (Fig. 3). The QIC of this model is greater than that of the same model with age (see above;
 267 QIC=333.9 vs. QIC= 301).

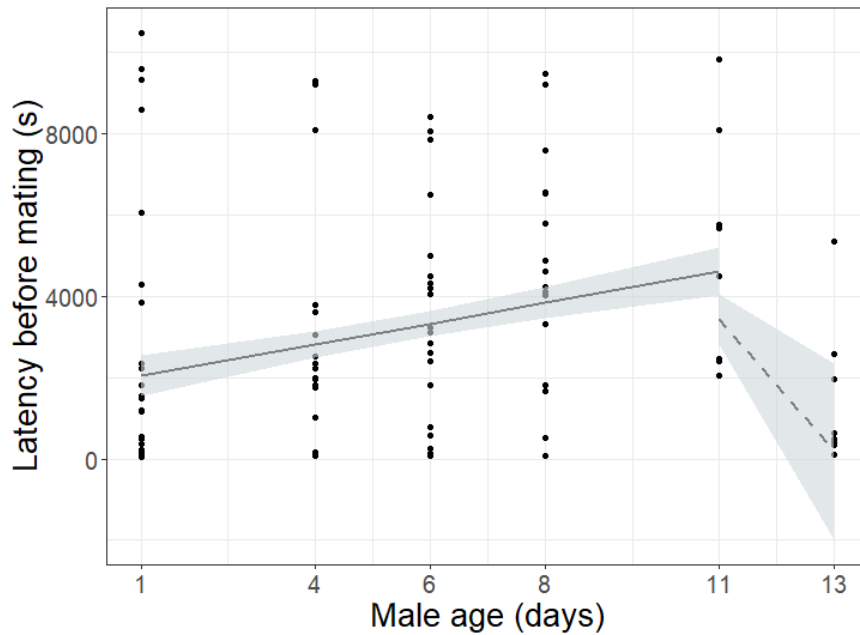


268

269 **Fig. 3** Male mating success (estimated by mating probability, see methods) according to the numbers
 270 of previous matings and the social environment (*Social* versus *Isolated* conditions). We represented
 271 model predictions and their confidence interval (coloured areas). Mating success = 0 indicates that
 272 male did not mate; mating success = 1 indicates that males mated. Dots represent mating observations
 273 in different numbers of matings of the male. In order to avoid overlapping dots, they have been spaced
 274 out in the figure

275 *ii) Effect of social environment on male reproductive behaviour*

276 First, we tested for an effect of the social environment on senescence in two male reproductive traits:
 277 latency to mate and copulation duration. Age of males influenced latency before mating ($\chi^2 = 10.15$, df
 278 = 2, $p = 0.006$). The best model that describes the age effect is a 2 slopes model without any effect of
 279 social environment (Appendix 2). Latency before mating increased between day 1 and day 11 but
 280 started to decrease after day 11 (Fig. 4, slope before 11 days \pm SE = 257 \pm 91 seconds, $\chi^2 = 7.44$, $df = 1$,
 281 $p = 0.006$; slope after 11 days \pm SE = -1625 \pm 578 seconds, $\chi^2 = 7.62$, $df = 1$, $p = 0.006$). In contrast,
 282 there was no effect of male age or the social environment on mating duration (Appendix 3).



283

284 **Fig. 4** Effect of male age on latency before mating. The selected model included two slopes with
 285 threshold (i.e. age at the onset of senescence) at 11 days. Model predictions are represented with their
 286 confidence interval (grey area). Dots represent latency for each mating observation

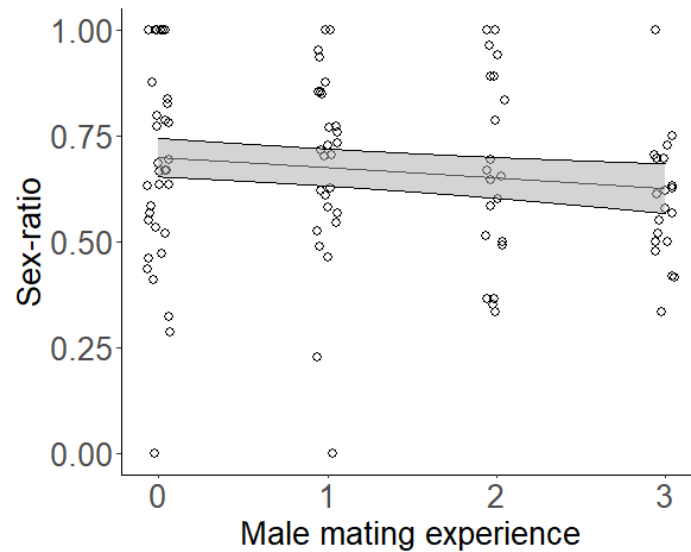
287 Second, we tested the effect of mating experience on those two reproductive traits according to the
 288 condition of social environment. Mating experience did not influence latency before mating ($\chi^2 = 0.04$,
 289 $df = 1$, $p = 0.84$) nor the mating duration ($\chi^2 = 0.55$, $df = 1$, $p = 0.46$). Social environment did not
 290 impact either of these two reproductive characteristics (latency: $\chi^2 = 0.008$, $df = 1$, $p = 0.93$; duration:
 291 $\chi^2 = 2.35$, $df = 1$, $p = 0.99$).

292 *iii) Effect of social environment on offspring production capacity*

293 Offspring production capacity by males (estimated by sex-ratio produced by each mated female) did
 294 not vary as a function of male age nor social environment treatment (age: $\chi^2 = 1.18$, $df = 1$, $p = 0.28$;
 295 social environment: $\chi^2 = 2.74$, $df = 1$, $p = 0.098$, small effect size: Hedges' $g = 0.29$, $IC = [-0.10$;
 296 $0.68]$).

297 However, offspring production capacity increased (i.e. the produced offspring sex-ratio decreased)
 298 with the number of previous matings ($\chi^2 = 7.47$, $df = 1$, $p = 0.006$; Fig. 5). Social environment did not
 299 influence sex-ratio ($\chi^2 = 2.5$, $df = 1$, $p = 0.11$).

300



301

302 **Fig. 5** Effect of male mating experience (i.e. the number of male previous matings) and social
303 environment on offspring production capacity (sex-ratio). The black line represents the predictions
304 from the model with random effect of males with its confidence interval. Sex-ratio = number of males
305 (sons) /number of offspring. Males *V. canescens* progeny being only composed of females, a lower
306 sex-ratio indicates an higher offspring production for the males. In order to avoid overlapping dots,
307 they have been spaced out in the figure

308

309 Discussion

310 The effects of the social environment on reproductive behaviours can occur when males
311 compete with other males for access to resources or mating opportunities, or when males are
312 confronted with rivals outside the episodes of access to females (Chen and Sokolowski 2022; Harrison
313 et al. 2024). Our study deals with the second category and reveals that a male's social environment
314 over the life course affects both their longevity and mating success. Our results are summarized in
315 Table 1.

316 **Table 1** Effect of male social environment, age, mating experience and size on different
317 response variables (Y): male longevity or reproductive characteristics (mating probability, mating

| Explanatory variable Response variable | Male Size | Social Environment (SE) | Male Age | Male Mating Experience (Exp) | SE:Age | SE:Exp |
|---|------------------|--------------------------------|-----------------|-------------------------------------|---------------|---------------|
| Longevity | NS | *(-) | ∅ | NS | ∅ | NS |
| Mating Probability | NS | / | / | / | *(-) | *(-) |

318 latency, mating duration or offspring production capacity). Each column corresponds to an
319 explanatory variable (X) and each row corresponds to a response variable tested (Y). Male age and
320 male mating experience (i.e. the number of previous matings) were not tested in a single model due to
321 their correlation (see Methods). NS = non-significant effect, *(+) = significant positive effect, *(-) =
322 significant negative effect, *(+ → -) = significant positive then negative effect after 11 days, ∅ =
323 untested effect, / = effect not tested because the explanatory variable is part of a significant interaction
324 discussed in the text. SE = social environment, Exp = male mating experience and SE:Age and
325 SE:Exp correspond respectively to the effect of the interaction between social environment and male
326 age or mating experience. The effect sizes associated to the statistics and p-values are indicated in the
327 text

| | | | | | | |
|--------------------------------------|----|----|---------|------|----|----|
| Mating Latency | NS | NS | *(+ →-) | NS | NS | NS |
| Mating Duration | NS | NS | NS | NS | NS | NS |
| Offspring Production Capacity | NS | NS | NS | *(+) | NS | NS |

328

329 As expected, we show that longevity tends to be reduced when males are in the presence of
330 other males before each mating opportunity (*Social* condition) in comparison to the longevity of
331 isolated males. This result is in line with previous studies in insects (Price et al. 2012; Lizé et al. 2014;
332 Amiri and Bandani 2022). For example, without access to reproduction, males of the medfly *Ceratitis*
333 *capitata* have a 1.3-fold decrease in longevity for those living with 9 other male conspecifics
334 compared to males stored alone (Gaskin et al. 2002). Up to now, in *V. canescens* reproductive
335 senescence has been documented but only in females with a strong decline of oviposition rate and
336 number of offspring produced each day with age (Metzger et al. 2008; Gomes et al. 2024). Our study
337 shows that reproductive senescence (measured by the age-specific decline in mating probabilities) also
338 occurs in males and, more importantly, is modulated by social environment. Indeed, mating
339 probabilities decrease with age in males under *Social* condition (by 10-fold for an 11-day-old male in
340 comparison with a one-day-old male) while it remains almost constant in males kept in isolation.
341 Among studies investigating the effect of social environment on male reproductive success, similar
342 patterns to those described here were observed in other male insects. For example *D. melanogaster*
343 males show a decrease in mating probability when exposed to a rival during their life (Bretman et al.
344 2013; Chen and Sokolowski 2022). However, when considering variation in the sex-ratio that may
345 influence the intensity of male-male competition, reproductive senescence pattern in offspring
346 production does not seem to vary. This is the case in *Tenebrio molitor*, where males that experienced
347 different sex-ratio conditions in the rearing population have the same reproductive senescence pattern
348 (Jehan et al. 2020). Thus, the effects of social environment on reproductive senescence in insects
349 appears to be species, context and trait-dependent.

350 From an evolutionary point of view, our results have implications for male reproductive
351 strategy. The deleterious effects of the social environment on male longevity should favour early
352 reproduction in male life. Similarly, the negative effects of social interactions between males on the
353 probability of mating, which increase with age, should select for early reproduction in males, and for
354 the ability to arrive fastest on rendez-vous sites to be the first to mate with females that are
355 monandrous (Metzger et al. 2010; Collet et al. 2020). This evolutionary scenario is compatible with
356 our hypothesis of interactions between males at rendez-vous sites. It will be now necessary to verify in
357 the field that these interactions at rendez-vous sites must take place early in the life of the males and
358 that young males arrive first at these sites.

359 Different mechanisms may explain the impact of the social environment on longevity and the
360 age-specific mating probabilities we detected. In general, a group of conspecific males may impose
361 constraints on focal males (e.g., higher intensity in competition for resources, such as food or mates).
362 Several studies support this hypothesis in terms of increased activity levels. When aggregated,
363 individuals (males and/or females) increase their level of activity in comparison with isolated ones
364 (e.g. in the field cricket *Gryllus integer*, Cade and Cade 1992; the grasshopper *Aiolopus thalassinus*,
365 Heifetz and Applebaum 1995; the cockroaches *Blattella germanica*, Lihoreau et al. 2009). Individuals
366 move more either to avoid contact with other individuals or to reduce the competition for resources
367 such as mates by dispersing to areas where competition is less intense. Males mustard leaf-beetles
368 *Phaedon cochleariae* reared in group as larvae or adults have a higher level of activity than males kept
369 isolated (Müller et al. 2016). This higher activity may lead to a greater energy expenditure resulting in
370 a shorter longevity and/or a lower mating opportunity, especially in late-life. In *Venturia canescens*,
371 we observed that the scramble competition occurring between males (Charrat et al. 2023) induces a
372 higher activity when in group – e.g. walking, rapid path changes or frequent flights. However, these
373 observations would require further more quantitative analyses. The fact that high energetic costs were
374 associated with flying in insects (causing up to a 50 to 100-fold increase in metabolic rate; for a review
375 see Beenackers et al. 1984) which is shown in *V. canescens* (Amat et al. 2012), could then explain the
376 shorter lifespan observed when males are in group. Alternatively, one may expect that males
377 experiencing contact with conspecifics allocate more resources towards the growth and maintenance

378 of postcopulatory traits (e.g. testes mass, Ramm and Stockley 2009). This could ultimately cause
379 fitness costs in terms of faster reproductive ageing (Lemaître et al. 2020). However, this hypothesis
380 seems unlikely in species such as *V. canescens* where females are monandrous and male benefits
381 through enhanced postcopulatory structures are limited.

382 In our study, the effect of time on mating probability is encompassed by two intertwined
383 parameters: male age or male mating experience. Experimentally, it is difficult to dissociate effects of
384 age and experience as the latter is acquired through successful and unsuccessful mating attempts
385 during an individual's life. The relative effect of male age and mating experience on mating and
386 reproductive success is rarely investigated and is still debated, as no general pattern of results emerges
387 (Aich et al. 2021). Indeed, the results are species-dependent: for example in hide beetle, *Dermestes*
388 *maculatus*, male age but not mating experience influences mating probability (Jones and Elgar 2004).
389 On the other hand, male age and sexual experience increase their mating probability in the Mexican
390 fruit fly, *Anastrepha ludens* (Pérez-Staples et al. 2010). In our experiment, age of *V. canescens* males
391 and mating experience (approximated by the number of matings already performed) are, as expected,
392 positively correlated ($r = 0.83$). Even if QIC analysis indicates that age is a better explanatory factor
393 than mating experience of the mating probability, further work is needed to disentangle these two
394 characteristics of male life and precisely assess the impact of social environment on reproductive
395 senescence. This should be all more true in natural conditions where these two aspects of male life are
396 probably much less strongly correlated than under standardized laboratory conditions (particularly
397 according to operational sex ratio or population density).

398 In insects, the use of past social information may change (i) aggressiveness (e.g. in *D.*
399 *melanogaster* (Nandy et al. 2016; Bath et al. 2021)), (ii) reproductive behaviours (Bretman et al. 2011;
400 Dore et al. 2020; Chen and Sokolowski 2022) and/or (iii) offspring production (e.g. in *D.*
401 *melanogaster*, Bretman et al. 2009). In this study, we showed that throughout *V. canescens* male
402 lifetime, when matings occurred, their characteristics (latency to mate and mating duration) and male
403 capacity of offspring production were not impacted by social environment. This is also observed in *D.*
404 *pseudoobscura* or *D. melanogaster* (Price et al. 2012; Hopkins et al. 2019). There are several possible
405 explanations for absence of social environment effects. When mating under *Social* conditions, males

406 could have adapted their behavioural response to the level of competition perceived in the past,
407 informing on potential future difficulties to reproduce. As female *V. canescens* are monandrous
408 (Metzger et al. 2010; Collet et al. 2020), this lack of shift in copulatory behaviour in response to social
409 environment may be due to the fact that there is no risk of sperm competition reducing the advantage
410 linked to an increase in mating duration and sperm transfer. Indeed, the latency before mating shows a
411 two-slope pattern in *V. canescens* males highlighting a change in the form of the relationship between
412 latency to mate and male age. First, latency to mate increases with age, as expected under the
413 hypothesis of a reproductive senescence. In a context of male competition and advantage of the faster
414 male to mate the monandrous female (which is expected in this species, Charrat et al. 2023), this
415 higher latency to mate could thus decrease male lifetime reproductive success. Then, the latency
416 decreases rapidly from the 11th day. Capacity of offspring production in males also slightly increases
417 with male experience as suggested by the decreasing sex-ratio we detected (increased female offspring
418 production). Changes with age or experience in mating latency and capacity of offspring production in
419 males suggest a terminal investment starting at 11 days in males *V. canescens*. This terminal
420 investment, defined as an increase in reproductive effort when the residual reproductive value
421 decreases (Williams 1966; Clutton-Brock 1984), was observed in several insects species (Creighton et
422 al. 2009). In *Venturia canescens*, females experiencing a simulation of climatic deterioration increase
423 strongly their oviposition activities (Amat et al. 2006).

424 Although in line with previous results in *V. canescens* addressing age-specific courtship
425 (Charrat et al. 2023), age and experience dependencies in latency to mate and capacity of offspring
426 production in males are associated with small effect sizes in this study. Such a stability in daughter
427 production in male *V. canescens* may be explained by an equal allocation to sperm transfer for each
428 mating, whatever the social condition, male age or reproductive experience. A complementary
429 experiment (Appendix 4) focused on sperm count data support this hypothesis. We counted as many
430 spermatozoa in ejaculates of experienced males, i.e. having already mated, as in inexperienced males,
431 i.e. mated for the first time. Whether social environment impacts sperm production or allocation
432 remains to be tested.

433 To conclude, we highlight an impaired longevity and a marked decrease in mating success
434 with male age when males encounter other males throughout their lives. These effects of social
435 environment are not accompanied by a change in male reproductive behaviour but the presence of
436 conspecifics may increase activity levels through physical interactions between rivals, as it has been
437 observed in groups of *V. canescens* males (B.C. pers. obs.). This might result in a greater energy
438 expenditure and thus a decrease in longevity and mating success. Further work is now needed to
439 explore the effect of present information related to social environment (i.e. number of rivals during a
440 mating opportunity) in male *Venturia canescens* and the potential influence of female behaviours in
441 interactions with the group of males.

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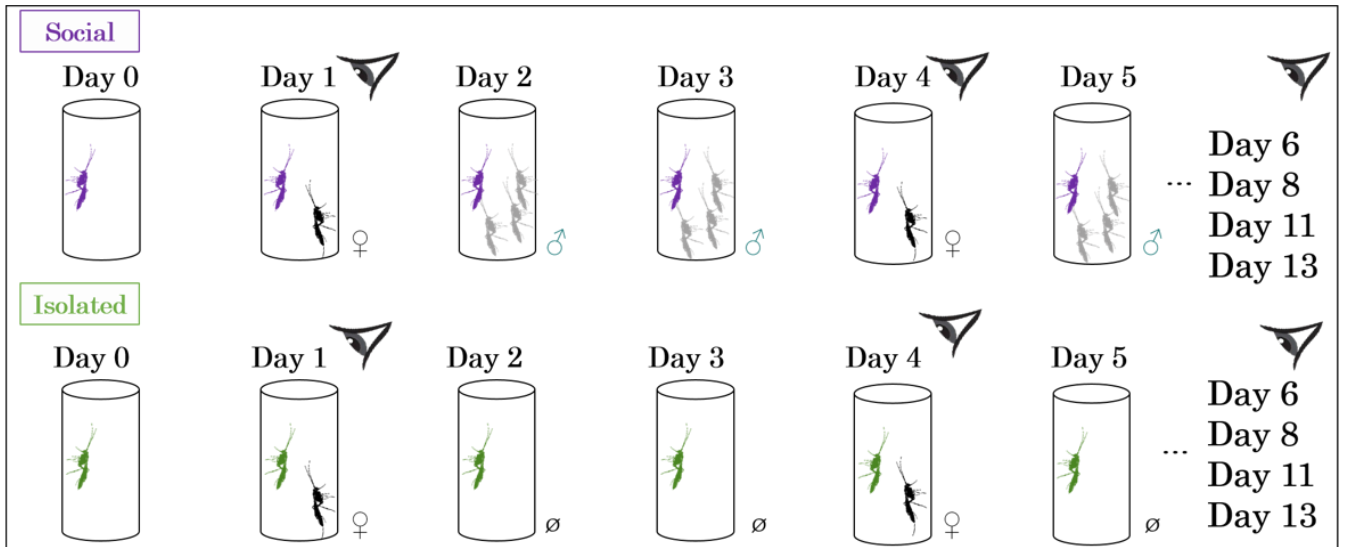
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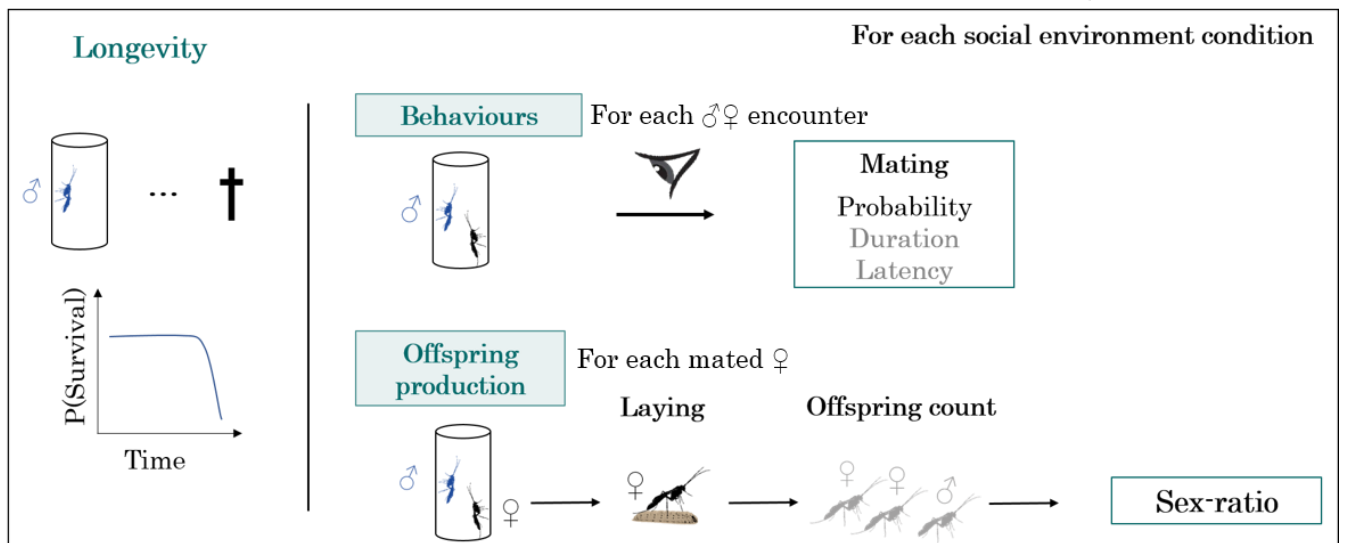
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610

A: Experimental planning



B: Response variables measured



613

614 **Fig. 6** Global diagram of the experimental protocol (A) and response variables measured (B). Focal
 615 males encountered one virgin female on days 1, 4, 6, 8, 11 and 13. Focal males from *Social* condition
 616 encountered 3 non-focal males on days 2, 3, 5, 7, 9, 10 and 12. After Day 13 (last possible male-
 617 female encounter), males were kept alone with food until they died: then, their longevity was
 618 calculated as the median between the last time they were seen alive and the first time they were seen
 619 dead. The eye represents the moments where individuals were observed and behaviours (mating
 620 characteristics) were measured for each male-female encounter. Offspring production from a mating

621 event of a male was measured by counting the number of sons and daughters laid by the mated female
622 and calculating the sex-ratio.

623

624 **Appendix 2**

625 **Model selection for the effect of male age and social environment on mating latency**

626 In Table 2, we presented values of AICc for each possible model aiming to identify the effects of male
 627 age and social environment (and their interaction) on mating latency. In bold is indicated the best
 628 model (*i.e.* the one with the lower AICc).

629 **Table 2** AICc of models describing the effect of male age, social environment and their interaction on
 630 mating latency.

| Type | Model | AICc |
|---------------------------------------|---|--------|
| constant then slope, threshold = 13 d | mating_latency~male_size+male_age.1*soc_envt+(1 ID_male) | 1966.3 |
| | mating_latency~male_size+male_age.1+soc_envt+(1 ID_male) | 1966.3 |
| | mating_latency~male_size+soc_envt+(1 ID_male) | 1966.3 |
| | mating_latency~male_age.1*soc_envt+(1 ID_male) | 1964.7 |
| | mating_latency~male_age.1+soc_envt+(1 ID_male) | 1964.7 |
| | mating_latency~soc_envt+(1 ID_male) | 1964.7 |
| | mating_latency~male_size+male_age.1+(1 ID_male) | 1964.2 |
| | mating_latency~male_size+(1 ID_male) | 1964.2 |
| | mating_latency~male_age.1+(1 ID_male) | 1962.6 |
| slope then constant, threshold = 8 d | mating_latency~male_size+soc_envt+(1 ID_male) | 1966.3 |
| | mating_latency~male_size+male_age.2*soc_envt+(1 ID_male) | 1965.5 |
| | mating_latency~male_size+male_age.2+soc_envt+(1 ID_male) | 1965.2 |
| | mating_latency~soc_envt+(1 ID_male) | 1964.7 |
| | mating_latency~male_size+(1 ID_male) | 1964.2 |
| | mating_latency~male_age.2*soc_envt+(1 ID_male) | 1963.5 |
| | mating_latency~male_age.2+soc_envt+(1 ID_male) | 1963.3 |
| | mating_latency~male_size+male_age.2+(1 ID_male) | 1963.1 |
| | mating_latency~male_age.2+(1 ID_male) | 1961.2 |
| 2 slopes, threshold = 11 d | mating_latency ~ male_size+male_age.2*soc_envt+(1 ID_male) | 1968 |
| | mating_latency ~ male_size+male_age.1*soc_envt +(1 ID_male) | 1967 |
| | mating_latency ~ male_age.2*soc_envt +(1 ID_male) | 1966.5 |

| | | |
|-----------------|--|---------------|
| | mating_latency ~ male_size+ soc_envt +(1 ID_male) | 1966.3 |
| | mating_latency ~ male_size+male_age.1+soc_envt +(1 ID_male) | 1966.1 |
| | mating_latency ~ male_size+ male_age.2+soc_envt +(1 ID_male) | 1965.7 |
| | mating_latency ~ male_age.1*soc_envt +(1 ID_male) | 1965.1 |
| | mating_latency ~ soc_envt+(1 ID_male) | 1964.7 |
| | mating_latency ~ male_age.1+soc_envt +(1 ID_male) | 1964.3 |
| | mating_latency ~ male_age.2+soc_envt+(1 ID_male) | 1964.2 |
| | mating_latency ~ male_size+(1 ID_male) | 1964.2 |
| | mating_latency ~ male_size+male_age.1+(1 ID_male) | 1964.1 |
| | mating_latency ~ male_size+male_age.2+(1 ID_male) | 1963.5 |
| | mating_latency ~ male_size+male_age.1+soc_envt+ male_age.2*soc_envt +(1 ID_male) | 1963.2 |
| | mating_latency ~ male_size+male_age.1*soc_envt+ male_age.2*soc_envt +(1 ID_male) | 1962.9 |
| | mating_latency ~ male_age.1+(1 ID_male) | 1962.2 |
| | mating_latency ~ male_age.2+(1 ID_male) | 1962 |
| | mating_latency ~ male_age.1+soc_envt+ male_age.2*soc_envt +(1 ID_male) | 1961.3 |
| | mating_latency ~ male_size+male_age.1*soc_envt+ male_age.2+soc_envt +(1 ID_male) | 1961.1 |
| | mating_latency ~ male_size+male_age.1+soc_envt+ male_age.2 +(1 ID_male) | 1960.8 |
| | mating_latency ~ male_age.1*soc_envt+ male_age.2*soc_envt +(1 ID_male) | 1960.8 |
| | mating_latency ~ male_age.1*soc_envt+ male_age.2+soc_envt +(1 ID_male) | 1959.1 |
| | mating_latency ~ male_age.1+ male_age.2+soc_envt +(1 ID_male) | 1959 |
| | mating_latency ~ male_size+male_age.1+ male_age.2+(1 ID_male) | 1958.7 |
| | mating_latency ~ male_age.1+ male_age.2+(1 ID_male) | 1956.8 |
| quadratic model | mating_latency ~male_size+I(male_age^2)*soc_envt+(1 ID_male) | 1970 |
| | mating_latency ~male_size+male_age+soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1968.5 |
| | mating_latency ~male_size+male_age*soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1968.5 |
| | mating_latency ~male_size+male_age*soc_envt+(1 ID_male) | 1968.5 |
| | mating_latency ~I(male_age^2)*soc_envt+(1 ID_male) | 1968.2 |
| | mating_latency ~male_size+I(male_age^2)+soc_envt+(1 ID_male) | 1968.1 |
| | mating_latency ~male_size+male_age*soc_envt+I(male_age^2)+soc_envt+(1 ID_male) | 1967.4 |
| | mating_latency ~male_size+male_age+I(male_age^2)+soc_envt+(1 ID_male) | 1967.2 |
| | mating_latency ~male_size+male_age+soc_envt+(1 ID_male) | 1967.1 |
| | mating_latency ~male_age*soc_envt+(1 ID_male) | 1966.6 |

| | | |
|--------------|--|--------|
| | mating_latency ~male_age+soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1966.5 |
| | mating_latency ~I(male_age^2)+soc_envt+(1 ID_male) | 1966.4 |
| | mating_latency ~male_size+soc_envt+(1 ID_male) | 1966.3 |
| | mating_latency ~male_age*soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1966.3 |
| | mating_latency ~male_size+I(male_age^2)+(1 ID_male) | 1966 |
| | mating_latency ~male_age*soc_envt+I(male_age^2)+soc_envt+(1 ID_male) | 1965.4 |
| | mating_latency ~male_age+soc_envt+(1 ID_male) | 1965.4 |
| | mating_latency ~male_age+I(male_age^2)+soc_envt+(1 ID_male) | 1965.2 |
| | mating_latency ~male_size+male_age+(1 ID_male) | 1965.1 |
| | mating_latency ~male_size+male_age+I(male_age^2)+(1 ID_male) | 1965 |
| | mating_latency ~male_age+soc_envt+(1 ID_male) | 1964.7 |
| | mating_latency ~I(male_age^2)+(1 ID_male) | 1964.3 |
| | mating_latency ~male_size+(1 ID_male) | 1964.2 |
| | mating_latency ~male_age+(1 ID_male) | 1963.3 |
| | mating_latency ~male_age+I(male_age^2)+(1 ID_male) | 1963 |
| linear model | mating_latency~male_size+male_age*soc_envt+(1 ID_male) | 1968.5 |
| | mating_latency~male_size+male_age+soc_envt+(1 ID_male) | 1967.1 |
| | mating_latency~male_age*soc_envt+(1 ID_male) | 1966.6 |
| | mating_latency~male_size+soc_envt+(1 ID_male) | 1966.3 |
| | mating_latency~male_age+soc_envt+(1 ID_male) | 1965.4 |
| | mating_latency~male_size+male_age+(1 ID_male) | 1965.1 |
| | mating_latency~soc_envt+(1 ID_male) | 1964.7 |
| | mating_latency~male_size+(1 ID_male) | 1964.2 |
| | mating_latency~male_age+(1 ID_male) | 1963.3 |
| null model | mating_latency~1+(1 ID_male) | 1962.6 |

631

632 [Appendix 3](#)

633 **Model selection for the effect of male age and social environment on mating duration**

634 In Table 3, we presented values of AICc for each possible model aiming to identify the potential effect
635 of male age and social environment (and their interaction) on mating duration. In bold is indicated the
636 best model (*i.e.* the one with the lower AICc).

637 **Table 3** AICc of models describing the effect of male age, social environment and their interaction on
638 mating latency.

| Type | Model | AICc |
|---------------------------------------|---|--------|
| constant then slope, threshold = 13 d | mating_duration ~ male_size+male_age.1*soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~ male_size+male_age.1+soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~ male_size+soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~ male_age.1*soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~ male_age.1+soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~ soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~ male_size+male_age.1+(1 ID_male) | 1042.2 |
| | mating_duration ~ male_size+(1 ID_male) | 1042.2 |
| | mating_duration ~ male_age.1+(1 ID_male) | 1040.2 |
| slope then constant, threshold = 1 d | mating_duration ~ male_size+male_age.2*soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~ male_size+male_age.2+soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~ male_size+soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~ male_age.2*soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~ male_age.2+soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~ soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~ male_size+male_age.2+(1 ID_male) | 1042.2 |
| | mating_duration ~ male_size+(1 ID_male) | 1042.2 |
| | mating_duration ~ male_age.2+(1 ID_male) | 1040.2 |
| 2 slopes, threshold = 1 d | mating_duration ~male_size+male_age.1*soc_envt+ male_age.2*soc_envt+(1 ID_male) | 1047.7 |
| | mating_duration ~male_size+male_age.1+soc_envt+ male_age.2*soc_envt+(1 ID_male) | 1047.7 |
| | mating_duration ~male_size+soc_envt+ male_age.2*soc_envt+(1 ID_male) | 1047.7 |
| | mating_duration ~male_size+male_age.1*soc_envt+ male_age.2+soc_envt+(1 ID_male) | 1045.9 |
| | mating_duration ~male_size+male_age.1+ male_age.2+soc_envt+(1 ID_male) | 1045.9 |
| | mating_duration ~male_size+ male_age.2+soc_envt+(1 ID_male) | 1045.9 |

| | | |
|-----------------|---|--------|
| | mating_duration ~male_age.1*soc_envt+ male_age.2*soc_envt+(1 ID_male) | 1045.5 |
| | mating_duration ~male_age.1+soc_envt+ male_age.2*soc_envt+(1 ID_male) | 1045.5 |
| | mating_duration ~soc_envt+ male_age.2*soc_envt+(1 ID_male) | 1045.5 |
| | mating_duration ~male_size+male_age.1*soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~male_size+male_age.1+soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~male_size+soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~male_age.1*soc_envt+ male_age.2+soc_envt+(1 ID_male) | 1043.7 |
| | mating_duration ~male_age.1+ male_age.2+soc_envt+(1 ID_male) | 1043.7 |
| | mating_duration ~ male_age.2+soc_envt+(1 ID_male) | 1043.7 |
| | mating_duration ~ male_size+male_age.1+male_age.2+soc_envt+(1 ID_male) | 1043.7 |
| | mating_duration ~ male_size+male_age.2+soc_envt+(1 ID_male) | 1043.7 |
| | mating_duration ~male_age.1*soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~male_age.1+soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~male_size+male_age.1+(1 ID_male) | 1042.2 |
| | mating_duration ~male_size+(1 ID_male) | 1042.2 |
| | mating_duration ~male_age.1+ male_age.2+(1 ID_male) | 1041.5 |
| | mating_duration ~ male_age.2+(1 ID_male) | 1041.5 |
| | mating_duration ~ male_age.1+(1 ID_male) | 1040.2 |
| quadratic model | mating_duration ~male_size+male_age*soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1052.4 |
| | mating_duration ~male_size+male_age+soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1050.2 |
| | mating_duration ~male_age*soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1050.1 |
| | mating_duration ~male_size+male_age*soc_envt+I(male_age^2)+soc_envt+(1 ID_male) | 1050.1 |
| | mating_duration ~male_size+male_age+I(male_age^2)+soc_envt+(1 ID_male) | 1048.1 |
| | mating_duration ~male_size+I(male_age^2)*soc_envt+(1 ID_male) | 1048 |
| | mating_duration ~male_age+soc_envt+I(male_age^2)*soc_envt+(1 ID_male) | 1047.9 |
| | mating_duration ~male_age*soc_envt+I(male_age^2)+soc_envt+(1 ID_male) | 1047.8 |
| | mating_duration ~male_size+male_age*soc_envt+soc_envt+(1 ID_male) | 1047.7 |
| | mating_duration ~male_size+I(male_age^2)+soc_envt+(1 ID_male) | 1046.1 |
| | mating_duration ~male_size+male_age+soc_envt+(1 ID_male) | 1045.9 |
| | mating_duration ~male_age+I(male_age^2)+soc_envt+(1 ID_male) | 1045.9 |
| | mating_duration ~male_size+male_age+I(male_age^2)+(1 ID_male) | 1045.8 |

| | | |
|--------------|---|---------------|
| | mating_duration ~I(male_age^2)*soc_envt+(1 ID_male) | 1045.8 |
| | mating_duration ~male_age*soc_envt+(1 ID_male) | 1045.5 |
| | mating_duration ~male_size+soc_envt+(1 ID_male) | 1044.4 |
| | mating_duration ~I(male_age^2)+soc_envt+(1 ID_male) | 1043.9 |
| | mating_duration ~male_size+I(male_age^2)+(1 ID_male) | 1043.9 |
| | mating_duration ~male_age+soc_envt+(1 ID_male) | 1043.7 |
| | mating_duration ~male_size+male_age+(1 ID_male) | 1043.7 |
| | mating_duration ~male_age+I(male_age^2)+(1 ID_male) | 1043.6 |
| | mating_duration ~soc_envt+(1 ID_male) | 1042.3 |
| | mating_duration ~male_size+(1 ID_male) | 1042.2 |
| | mating_duration I(male_age^2)+(1 ID_male) | 1041.7 |
| | mating_duration ~male_age+(1 ID_male) | 1041.5 |
| linear model | mating_duration ~ male_size+male_age *soc_envt+ (1 ID_male) | 1047.7 |
| | mating_duration ~ male_size+male_age +soc_envt+ (1 ID_male) | 1045.9 |
| | mating_duration ~ male_age *soc_envt+ (1 ID_male) | 1045.5 |
| | mating_duration ~ male_size+soc_envt+ (1 ID_male) | 1044.4 |
| | mating_duration ~ male_age +soc_envt+ (1 ID_male) | 1043.7 |
| | mating_duration ~ male_size+male_age+ (1 ID_male) | 1043.7 |
| | mating_duration ~ soc_envt+ (1 ID_male) | 1042.3 |
| | mating_duration ~ male_size+ (1 ID_male) | 1042.2 |
| null | mating_duration ~ 1+(1 ID_male) | 1040.2 |

639

640 [Appendix 4](#)

641 **Male mating experience and ejaculate size**

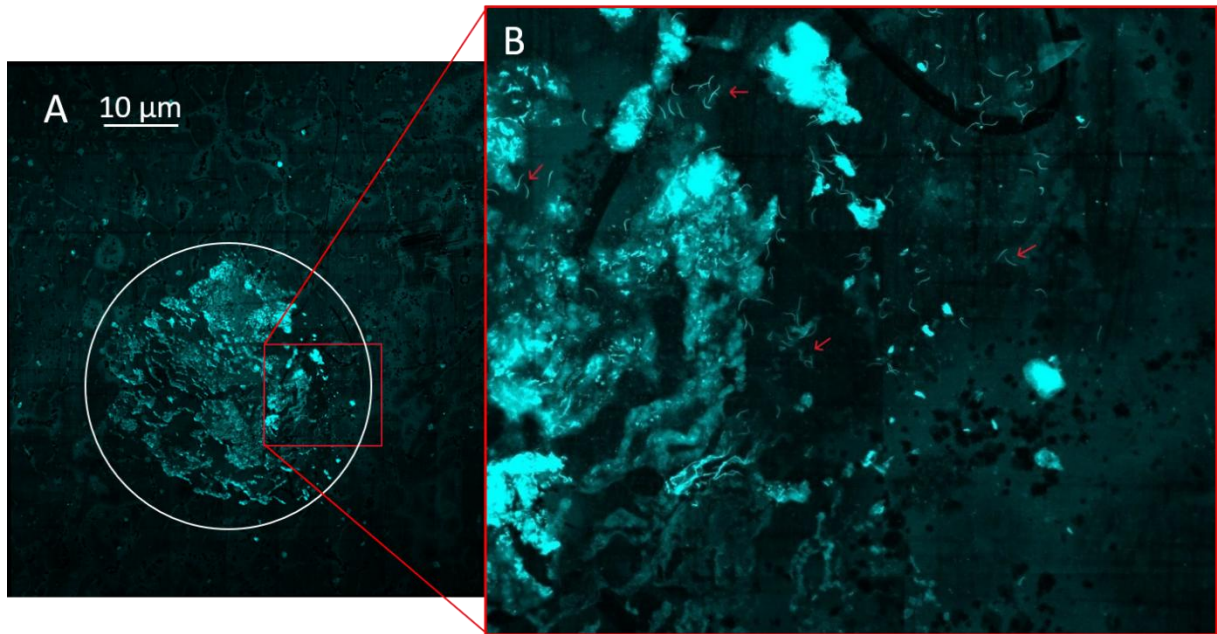
642 A complementary experiment was designed to test whether males' multiple mating
643 experiences influenced the size of their ejaculates, estimated by the number of spermatozoa stored in
644 the spermatheca, and therefore the number of spermatozoa that females could use to produce
645 daughters. We predicted that the size of the ejaculate is smaller in males that have already experienced
646 several matings (referred hereafter as “Experienced” males) than in males mating for the first time
647 (“Inexperienced” males).

648 At emergence, males were randomly assigned to the “Experienced” or “Inexperienced” population
649 cages, the former containing both males and females while the later containing only males (all
650 individuals were randomly drawn from rearing boxes). Density within the cages varied slightly
651 between treatments and replicates according to the number of males and females emerging each week.
652 Individuals of both treatments were provided with food (honey) *ad libitum*.

653 At the end of the third day, females were removed from the “experienced” cages. On day 4 morning,
654 food was removed from the cages, and 2h later, as many one-day-old virgin females as males (already
655 in the cages) were introduced in “Experienced” and “Inexperienced” cages, respectively. During 2
656 hours, we observed and recorded matings. Each couple in copulation was gently withdrawn from the
657 cage and kept in a tube. Then, mated females were dissected (from 2 to 24 hours post mating) and
658 spermatozoa transferred by males (i.e. stored in spermatheca) were counted with ImageJ software
659 (Schneider et al. 2012) by experimenters who were blind to the experimental treatment
660 (“Inexperienced” vs (“Experienced” groups). Thirty-seven and 32 matings occurred in the
661 “Inexperienced” and “Experienced” treatments respectively, but due to technical problems during
662 female dissections, 35 “Inexperienced” and 31 “Experienced” measurements only were included in the
663 dataset. The size of mated males was measured. All details regarding the dissection procedure are
664 provided below.

665 **Detailed protocol for the dissection of spermatheca and counting of spermatozoa**

666 Females were dissected in one drop of PBS1X and spermatheca was isolated and transferred on
667 another microscope glass slide with one drop of PBS1X/DAPI, DAPI diluted 1:1.000 (DAPI,
668 Thermoscientific, reference 6224B). Spermatheca was crushed between the slide and a cover glass by
669 thumb pressure on the cover glass. Cover glasses were sealed with varnish and stored at 4°C. An
670 image of each slide was taken with a confocal microscope (Fig. 7, Zeiss, LSM 800, magnification
671 x20).



672

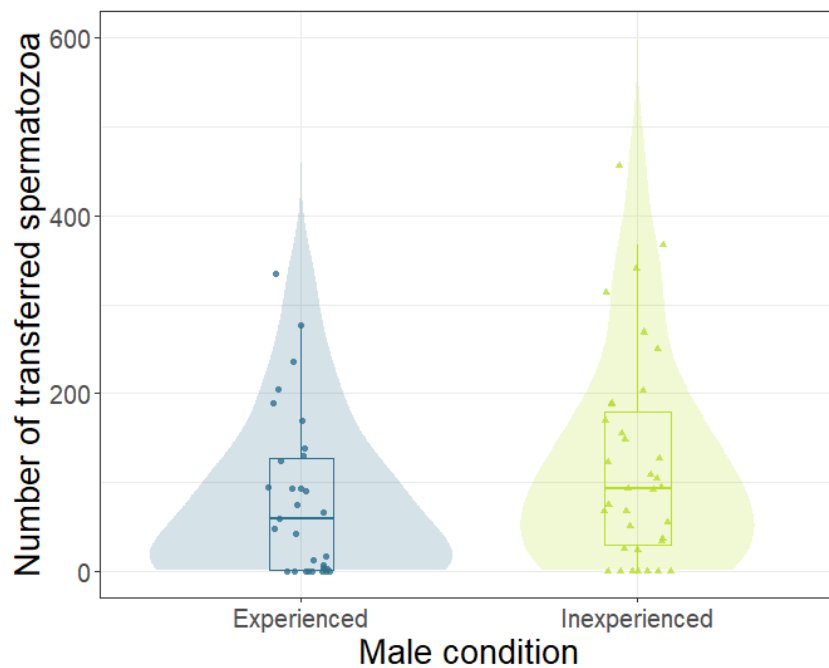
673

674 **Fig. 7** Spermatheca and spermatozoa coloured with DAPI between slide and cover glass, under
675 confocal microscope. A - Image of the whole cover glass. Crushed spermatheca is indicated by the
676 white circle. B - Cropped image corresponding to the red square in image A. Red arrows show
677 examples of spermatozoa

678 **Results - Effect of male mating experience on spermatozoa transfer**

679 We first compared the number of spermatozoa counted by the two experimentalists with a Wilcoxon
680 signed-rank test. As there was no difference ($W = 458$, $p = 0.3$), we retained the lowest value between
681 the two experimentalists for further analysis (we draw qualitatively the same conclusions whatever the
682 metric used: median sperm count or largest value). We also checked that there was no influence of the
683 time since mating on spermatozoa number in the spermatheca ($t = -0.06$, $df = 28$, $p = 0.95$). The effect
684 of male mating experience on the number of spermatozoa transferred was tested with a zero-inflated
685 Poisson model, with Poisson distribution and the identity of the cage in which males were as random
686 factor (*glmmTMB* packages Brooks et al. 2017). This allowed us to consider the differences in density
687 between cage. As there were many zeros in the data which caused overdispersion, we used zero-
688 inflated model in order to account for this.

689 We did not detect an effect of male mating experience, i.e. if the male could mate before the
690 experiment or not, on the size of the ejaculate (Fig. 8). Indeed, male experience (“Experienced” and
691 “Inexperienced”) did not influence the number of transferred spermatozoa in female spermatheca (χ^2
692 =0.713, df = 1, p = 0.398).



693

694

695 **Fig. 8** Effect of male mating experience on the number of transferred spermatozoa stored in the
696 spermatheca. Each dot represents number of spermatozoa for one mating. Violin plots represent the
697 probability density

698