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Karen Muller, Denis Thiéry, Sébastien Motreuil, Jérôme Moreau

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1 **What makes a good mate? Factors influencing male and female**
2 **reproductive success in a polyphagous moth**

3 The mating propensity of an individual is expected to depend on the costs and benefits of
4 mating, which may vary across the sexes and across different mating opportunities. Both
5 males and females should gain fitness either by mating with multiple mates and/or by mating
6 with higher quality mates. Therefore, an important question in the area of sexual selection
7 concerns what makes an optimal mate. From a female perspective, females are expected to
8 prefer males providing direct material benefits for the present generation and/or indirect
9 genetic benefits for their offspring in the subsequent generation. Because male contribution to
10 these benefits can be limited, as reproduction imposes non-trivial costs on males, female
11 benefits from mating can vary markedly as a function of the condition of their mate. In capital
12 breeding species, in which males invest the majority of their larval resources in a single
13 reproductive event, the females are likely to prefer to mate with virgin males in good
14 condition (i.e. males that have developed on high quality food sources). In this study we used
15 the European grapevine moth (*Lobesia botrana*) to test experimentally whether the larval
16 nutrition and mating history of males influence their quality as mates. We provided wild *L.*
17 *botrana* males originating from different cultivars and vineyards with unlimited access to
18 standardized females, and examined the lifetime reproductive success of the males and the
19 consequences for the reproductive output of females. Our results show that ‘male quality’
20 depended on both the male larval origin and mating history, and that females discriminated
21 among males and mated more with males having high spermatophore quality (virgin males
22 and males from certain cultivars or vineyards) to obtain substantial direct benefits.

23 **KEY WORDS:** direct benefits, larval nutrition, *Lobesia botrana*, male mating history, male
24 quality, spermatophore.

25 The mating propensity of an individual is expected to depend on the costs and benefits of
26 mating, which may vary across the sexes and the number of mating opportunities. When both
27 male and female vary in their reproductive quality, the two sexes are expected to be choosy
28 and should display higher mating preferences with partners providing higher fitness benefits.
29 Males and females should gain fitness either by mating with multiple mates (Arnqvist &
30 Nilsson 2000, Wagner et al. 2011) and/or by mating with higher quality mates. Therefore, an
31 important question in the sexual selection area concerns what makes an optimal mate for the
32 choosy sex. 'Mate reproductive quality' is determined by a variety of behavioral,
33 physiological and morphological traits (Lailvaux & Kasumovic 2010, Wilson & Nussey
34 2010). These traits influence the propensity to mate of individuals (through precopulatory
35 behaviors including courtship, production of sex pheromone, and mate guarding) and
36 therefore influence their probability of being chosen as a mate and shape their realized fitness
37 (Simmons 2001).

38 The benefits of mate choice depend on the quality of the chosen mate but also on the extrinsic
39 and intrinsic conditions of the choosy individual, including its physiological state and physical
40 and social environment. For example, some studies have shown that males mate preferentially
41 with more fecund females (Bonduriansky 2001) and tailor their ejaculate size to the level of
42 sperm competition (Wedell et al. 2002). In the same way, female mating behaviour is affected
43 by a variety of intrinsic (including mating status or age) and extrinsic factors (such as
44 predation risk, parasite infection or mate availability). Because females that fail to mate have
45 zero fitness (Rhainds 2010), the level of female choosiness is constrained by the risk of
46 remaining unmated, which depends on demographic effects, low mate encounter rate, out-
47 competition by rivals or prereproductive death (Kokko & Mappes 2005, Rhainds 2010,
48 Rhainds 2013). Thus, female mating strategies often reflect a trade-off between maximizing
49 the benefits of obtaining high quality mates, reducing the probability of mating failure and

50 minimizing other mating costs (Rhainds 2010). By keeping in mind these trade-offs, good
51 mates for females are those that are able to provide direct and indirect benefits (Møller &
52 Jennions 2001). Indirect benefits can arise from genetic traits of the chosen male (e.g. good
53 genes), which lead to increased fitness of the resulting offspring (Mays & Hill 2004, Tregenza
54 & Wedell 2000). Direct benefits are related to whether the chosen male is sufficiently fertile,
55 free of disease, or able to provide parental care, access to territories or to nutritive resources
56 including nuptial gifts (Choe & Crespi 1997, Vahed 1998). However, the male contribution to
57 these direct benefits can be limited, as reproduction imposes non-trivial costs on males,
58 arising from mate location, competition, courtship, parental care, and especially ejaculate
59 production (Janowitz & Fischer 2010, Paukku & Kotiaho 2005, Scharf et al. 2013). Thus,
60 female benefits from mating can be extremely variable based on the quality of their mate,
61 because factors limiting the reproduction of males can have profound consequences for
62 female reproductive output.

63 For species in which males provide females with material resources including a nutritive
64 ejaculate (for example, spermatophores in some lepidopteran species), the influence of male
65 mating frequency on future reproductive output can also be extremely pronounced (Torres-
66 Vila & Jennions 2005, Wedell et al. 2002). Because ejaculate production is costly (Dewsbury
67 1982), male performance usually declines across multiple matings, leading to diminishing
68 reproductive returns for males (reviewed by Simmons 2001). Moreover, males may be limited
69 in the amount of sperm they can transfer to a female during mating (Marcotte et al. 2005), and
70 male mating history (the number of previous matings) is certainly a key factor determining
71 female fitness, especially in species in which males can keep copulating despite being sperm
72 depleted (Damiens & Bovin 2006, Steiner et al. 2008). It has been commonly assumed that
73 males have to face trade-offs between an investment in somatic maintenance or in
74 reproduction because they have finite resources to invest (Barnes & Partridge 2003, Stearns

75 1992). Such trade-offs typically arise under food limitation, because male expenditure in
76 ejaculate production is constrained in part by resource availability; consequently males have
77 to invest in either current or future reproduction (Simmons 2001). In capital breeders, which
78 rely mainly on larval reserves for successful reproduction, the resources needed to produce a
79 nutritive ejaculate can be a limiting factor. Therefore, ejaculate production could be related to
80 the number of copulations and male larval nutrition, but few studies have reported the
81 quantitative and qualitative relationships involved. Diet quality can have a significant
82 influence on the rate at which males produce ejaculate, the quality of the seminal fluid
83 proteins, and the effectiveness of the ejaculate in achieving fertilization (Arnqvist &
84 Danielsson 1999, Gage & Cook 1994, Simmons & Kvarnemo 1997). When males lack
85 adequate protein sources or when they have developed on nutritionally limited host plants,
86 critical depletion of their ejaculate generally occurs during successive matings (Gage & Cook
87 1994). However, most studies have focused on the factors affecting male reproductive output
88 following emergence, particularly during the first two mating events (Delisle & Hardy 1997,
89 Tigreros 2013, Cordes et al. 2015) but not on the trade-off between larval nutrition and the
90 male entire lifetime reproductive investment.

91 In this context, our study goals were to assess, if (1) larval nutrition has an overall importance
92 in male mating capacity and lifetime reproductive investment; (2) the combined effect of male
93 larval nutrition and male mating history affect their quality as mates and (3) female prefer to
94 mate with 'high quality mates' in order to obtain larger direct benefits. To answer these
95 questions, we used the European grapevine moth (*Lobesia botrana*; Lepidoptera: Tortricidae)
96 (Denis & Schiffermüller 1775), which is a very important pest of grapes worldwide. Several
97 studies of this species have already shown marked effects of larval nutrition on male and
98 female fitness (Moreau et al. 2006, 2007, Muller et al. 2015). However, the lifetime
99 reproductive capacity of male moths of this species remains unknown because most studies

100 have concerned only the first mating of individuals (Moreau et al. 2006, 2007, Muller et al.
101 2015; see Torres-Vila et al. 1999 for an exception). In the present study, we provided wild *L.*
102 *botrana* males that developed on different grape cultivars and in different vineyards with
103 unlimited access to females and investigated the lifetime reproductive success of the males.
104 We also investigated the consequences for the reproductive output of females as a function of
105 male larval origin and mating history. In a first step we explored variation in male
106 reproductive investment (spermatophore size, number of sperm) during successive matings.
107 We predicted that (i) male reproductive investment and mating capacity would be affected by
108 male larval nutrition on different cultivars; and (ii) male quality would depend on both their
109 larval nutrition and mating history. In a second step, we studied the consequences of male
110 larval nutrition and mating history on the reproductive output of females (fecundity and
111 fertility). We predicted (iii) that female fitness would be affected by both male larval origin
112 and mating history and (iv) that female would be more motivated to mate with males of ‘high
113 quality’, thus receiving larger nutrient-rich spermatophores.

114 **MATERIAL & METHODS**

115 *Field sampling*

116 *Lobesia botrana* is a major pest of grapes. It is widely distributed in most European vineyards
117 and is now present in the USA, where 3–4 larval generations occur each year, depending on
118 latitude. First generation larvae of *L. botrana* were collected in the field during June 2013. To
119 test for a cultivar effect within a given population, larvae were sampled from three grape
120 (*Vitis vinifera*) cultivars (‘Carignan’, ‘Mourvèdre’ and ‘Grenache’) in the same vineyard
121 (Perpignan, France; N 42°44′7.063", E 2°52′56.441"), ensuring the same abiotic conditions
122 (temperature, light exposure, humidity) for larval development. The three chosen grape
123 varieties are biochemically very different, especially in their phenolic contents (Teissedre and

124 Chervin 2011). Indeed, ‘Carignan’ and ‘Grenache’ grape extracts contains less total phenols
125 than Mourvèdre grape extracts (Jensen et al. 2008). To test for a geographical effect we
126 sampled larvae from the cultivar ‘Grenache’ from two additional geographically distinct
127 French vineyards; Estézargues (N 43°56'49.781", E 4°39'39.372") and Sénas
128 (N 43°43'54.251", E 5°1'45.621"). Larvae were sampled at the end of the larval cycle (fifth
129 instar), following construction of glomerulae made of flower buds aggregated in larval silk
130 (phenology 17–25; Eichhorn & Lorenz 1977). Larvae usually complete their development in a
131 single grape bunch, and each glomerulus is only occupied by a single larva (Torres-Vila et al.
132 1997). To collect newly emerged adults, larvae at the end of their development were placed in
133 large polyethylene boxes (60 × 40 cm, height 21 cm) in the laboratory and fed *ad libitum* on
134 grape bunches from the same cultivar and site where they developed, and were incubated at
135 22 ± 1 °C, 60 ± 10 % RH, and under natural photoperiod conditions. The larvae were checked
136 daily until pupation, at which time they were gently removed from their glomerulae. The
137 pupae were weighed to the nearest 0.1 mg using a Precisa 262 SMA-FR microbalance, placed
138 individually in glass tubes (70 × 9 mm diameter) stoppered with cotton wool plugs, and stored
139 at 22 ± 1 °C under natural photoperiod conditions. The pupae were checked each morning,
140 and newly emerged adults were visually sexed by examination of the ventral tip of the
141 abdomen.

142 To assess the importance of male larval origin and mating history on male reproductive
143 investment and female reproductive output, 2-day-old males of different larval origin (cultivar
144 and site) were given daily mating access to a new 1- or 2-day-old standardized virgin female;
145 this was continued until death of the male. The standardized females came from an inbred
146 strain (INRA Bordeaux) maintained without diapause on a semi-artificial diet. The use of this
147 inbred strain helps to minimize genetic variation between females and allows us to detect the
148 male effect on female reproduction (see Moreau et al. 2007 and Muller et al. 2015 for more

149 details). Males used for the following mating experiments were randomly distributed into two
150 subsamples. The first subsample was used to evaluate the effect of male origin (cultivar and
151 site) and mating history on male lifetime reproductive investment, and was also used to
152 monitor the male precopulatory behavior for each mating event. The second subsample was
153 used to assess the consequences of male origin (cultivar and site) and mating history on the
154 reproductive output of females.

155 *Ethical Note*

156 All experiments complied with French laws on animal experimentation. Moths were treated
157 carefully, and the abiotic conditions (temperature, humidity and photoperiod) they
158 experienced corresponded to the natural conditions in their native habitat. Females submitted
159 to dissection were chilled in a freezer prior to decapitation.

160 *Mating procedure*

161 At dusk, one male (2 days old on the first day of the experiment) randomly selected from each
162 test condition (cultivar or site) was placed into a mating tube (100 × 15 mm diameter) with a
163 single 1- or 2-day-old standardized virgin female, and the pair were observed until mating
164 took place, or for a maximum of 4 h in the absence of mating. The male was returned to the
165 pupation tube after mating or at 4 h, and held under the same conditions as for moth
166 maintenance, with water provided *ad libitum*. This process was repeated 24 h later in a new
167 mating tube, and the procedure was repeated sequentially until the death of the male. A
168 mating was considered to have been successful if a sperm-filled spermatophore was observed
169 in the bursa copulatrix after the dissection of the female under a stereomicroscope (Nikon
170 SMZ1500) at a magnification of 20×. Matings in which males failed to transfer a
171 spermatophore (if no spermatophore was found in the bursa copulatrix of the female, or if the

172 female lay no eggs during her life following the observed mating) were discarded from the
173 analysis. Various reproductive traits were measured in each subsample.

174 *First subsample: precopulatory behaviors of males and females*

175 For the first subsample the male and female sexual activity was videotaped (Sony HDR
176 CX220E) until mating; only recordings of successful matings (with effective spermatophore
177 transfer or female egg laying) were analyzed. The latency period prior to mating (the time
178 elapsed from male/female pairing until coupling) was recorded along with the occurrence of
179 behaviors reflecting female and male sexual motivation (as described by Muller et al. 2015).
180 The latency to mate is a first measure that accurately reflects the reluctance or acceptance to
181 mate in no-choice tests (Edward 2014, Muller et al. 2016). Moreover, in *L. botrana* a female
182 that is ready to mate signals readiness by releasing sex pheromone at dusk, which is an action
183 that represents a fitness cost (Harari et al. 2011). To do this the female assumes a calling
184 position with wings raised and the pheromone gland exposed. This behavior reflects the
185 penchant of a female to mate, and therefore we used it as a proxy of female motivation. Thus,
186 we recorded data on the female motivation to mate (expressed as the time a female spent
187 calling divided by the courtship duration $\times 100$). To evaluate mating ability and sexual vigor
188 of males in courtship, we also recorded data on the percentage of male activity (the time spent
189 in movement by the male expressed as a percent of the total courtship period).

190 *First subsample: male reproductive performance*

191 Immediately following mating the females were anesthetized (-25°C for 10 min), then
192 dissected on a glass slide. The bursa copulatrix containing the male spermatophore was
193 removed and measured. The spermatophore produced by *L. botrana* males is very small (< 1
194 mg) and consequently difficult to weigh accurately. We estimated the spermatophore size by
195 extrapolating its volume; this is a well-established method used for small moths including *L.*

196 *botrana* (Muller et al. 2015, Torres-Vila et al. 1999). The spermatophore length (l), width (w),
197 and thickness (t) were measured using a stereomicroscope (Nikon SMZ1500) at a
198 magnification of 20×, and the spermatophore volume was estimated as an ellipsoid balloon [V
199 $= \pi/6 (l \times w \times t)$], as described previously (Torres Vila et al. 1999, Muller et al. 2015). Like in
200 all Lepidoptera, male *L. botrana* transfer fertile eupyrene sperm and non-fertile anucleate
201 apyrene sperm at mating. The sperm-containing ampulla was ruptured in a drop of distilled
202 water and the sperm mass was gently stirred to ensure dispersion. In Lepidoptera at this stage
203 the eupyrene sperm are encysted in bundles, and each bundle contains 256 eupyrene sperm
204 (Cook & Gage 1995). The number of intact bundles was counted at 40×/0.65 magnification
205 using a Nikon Eclipse E600 microscope; this number was multiplied by 256 to estimate the
206 total number of eupyrene sperm. The solution was then washed from the slide into a 1.5 mL
207 centrifuge tube and diluted with distilled water. Four subsamples (10 μ L) were removed from
208 the diluted sperm solution, and the number of apyrene sperm was counted by microscopy
209 (Nikon Eclipse E600; 100× magnification). The total number of apyrene sperm was estimated
210 by multiplying the average sperm count for the four subsamples (coefficient of variation
211 = 12%) by the dilution factor.

212 We recorded: (i) male longevity; (ii) the total number of matings by males during their
213 lifespan; (iii) the lifetime spermatophore quantity produced and the lifetime number of sperm
214 transferred; (iv) the number of offspring sired by males during their lifetime; and (v) the
215 spermatophore volume and the number of sperm transferred at each male mating.

216 *Second subsample: consequences for female reproductive output*

217 Following mating (see general mating procedure) the females were held in the mating tube
218 and could oviposit freely on the inside surface of the glass tub. Female survival was checked
219 daily, and following death the eggs were incubated for 7 days under the same conditions as

220 for moth maintenance. We recorded several female traits as a function of male larval origin
221 (cultivar or site) and mating history, including: (i) female fecundity (the number of eggs laid
222 per female at each mating); and (v) female fertility (the proportion of hatched eggs for each
223 mating).

224 *Statistical analysis*

225 All statistical tests were performed using R Software version 3.2.0 (R Core Team 2015). For
226 each analysis we report the full model with insignificant interactions deleted, following the
227 approach of Forstmeier & Schielzeth (2011). The effect of male origin (cultivar and site) on
228 male pupal mass was tested using a one-way ANOVA followed by Tukey's test. A cox
229 regression was applied to assess the influence of male origin and pupal mass on male
230 longevity. Sources of variation in the total number of matings by males during their lifetime,
231 the lifetime quantity of spermatophore produced by males, and the number of offspring sired
232 by males during their lifetime were identified using ANCOVAs, with male larval origin as the
233 explanatory variable, and the number of male matings and the male and female pupal masses
234 as covariates. Because the sperm were counted and were over-dispersed, a generalized linear
235 model with a negative binomial distribution (NBGLM) was used to evaluate the effect of
236 male origin on the total number of sperm transferred by males during their lifetime.

237 We used a general mixed model with male identity as a random effect to assess the combined
238 effects of male mating history and larval origin on precopulatory behavior, the spermatophore
239 size, the number of eupyrene and apyrene sperm, and the female fecundity and fertility. Male
240 mating history was recorded as a discrete variable (1, 2, 3, 4, or 5 matings); the data for the
241 previous 5, 6 and 7 matings were excluded because the sample size was too small for certain
242 male origins (< 5 individuals). Pearson's χ^2 tests were used to assess the mating success of
243 males (percentage of successful matings) as a function of larval origin (cultivar and site) and
244 mating history. Because of non-normality, female motivation to mate (percent of time spent in

245 the calling position) and male activity (percent of time spent in movement) were arcsine
246 square root transformed prior to analysis. The latency period prior to mating, the male activity
247 and the female motivation to mate were analyzed using general linear mixed models. Because
248 data on sperm were best approximated by an over-dispersed Poisson distribution, we fitted the
249 model with a negative binomial error structure and used the glmmADMB library to perform
250 the analysis, which included male mating history and male larval origin as fixed effects, male
251 and female mass as covariates, and male identity as a random factor. The proportion of eggs
252 hatched was analyzed using the glmmPQL function with a quasi-binomial error structure.

253

254 **RESULTS**

255 *Male pupal mass and longevity*

256 Male pupal mass was affected by male larval origin (Table 1; $F_{4,181} = 20.63$, $P < 0.0001$).
257 Males from Mourvèdre in the Perpignan vineyard were larger than those from Carignan
258 (Table 1). Among sites, the males from Grenache in Estézargues and Sénas were heavier than
259 those from this cultivar in Perpignan. Male longevity was also influenced by the origin of the
260 males ($\chi^2_{4,181} = 23.23$, $P < 0.0001$), and was positively correlated with male pupal mass
261 ($\chi^2_{1,181} = 26.02$, $p < 0.0001$) (Table 1). At a given site (Perpignan), the males from Carignan
262 died earlier than males from Grenache and Mourvèdre. However, the longevity of males from
263 Grenache in Perpignan was similar to that of males from Grenache in Estézargues or Sénas.
264 As a consequence of differing longevity, males did not have the same number of mating
265 opportunities over their lifetimes. Consequently, male mating capacity (i.e. the maximum
266 number of matings that males undertook during their lifetimes) was positively correlated to
267 male longevity ($F_{1,179} = 188.01$, $P < 0.0001$). For example, in a given vineyard the males from
268 Carignan lived an average of 6 days, and tended to mate less often than males from Grenache
269 or Mourvèdre, which lived for more than 7 days (Table 1 and Fig. 1). Moreover, among the

270 males from Grenache in Sénas and Estézargues (which lived the longest), 50–75% mated 5 or
271 more times during their lifetime, while only 30% of the males from Grenache in Perpignan
272 mated at least 5 times (Table 1 and Fig. 1).

273 *Male lifetime reproductive investment*

274 The overall spermatophore quantity produced by males during their lifetime varied with the
275 total number of matings ($F_{1,73} = 146.36$, $P < 0.0001$), the male larval origin ($F_{4,73} = 9.76$,
276 $P < 0.0001$), and the pupal mass ($F_{1,73} = 17.86$, $P < 0.0001$) (Table 1). At a given site, males
277 from Grenache transferred a greater quantity of spermatophore to females at mating (average
278 $132.7 \times 10^{-6} \text{ mm}^3$) than males from Carignan (approximately $96.6 \times 10^{-6} \text{ mm}^3$) or Mourvèdre
279 (approximately $125 \times 10^{-6} \text{ mm}^3$). However, males from the three geographically distinct sites
280 transferred approximately the same quantities of spermatophore during their lifetimes. The
281 numbers of fertile eupyrene and non-fertile apyrene sperm were positively correlated with the
282 number of male matings ($F_{1,73} = 57.34$, $P < 0.0001$ and $F_{1,73} = 16.76$, $P < 0.0001$,
283 respectively), but were not correlated with the male larval origin ($F_{4,73} = 1.87$, $P = 0.124$ and
284 $F_{4,73} = 0.53$, $P = 0.713$, respectively) or the pupal mass ($F_{1,73} = 1.44$, $P = 0.234$ and $F_{1,73} = 1.17$
285 $P = 0.283$, respectively) (Table 1).

286 Consequently, the number of offspring derived from males during their lifetime was
287 influenced by the number of male matings and the male larval origin (Fig. 2; male mating
288 number effect: $F_{1,95} = 151.73$, $P < 0.0001$; male larval origin: $F_{4,95} = 21.46$, $P < 0.0001$;
289 interaction term: $F_{4,95} = 5.97$, $P = 0.001$), but not by the male pupal mass ($F_{1,95} = 1.25$,
290 $P = 0.266$). In a given vineyard, males from Mourvèdre always produced the least quantity of
291 spermatophore and consequently produced fewer offspring than males from the two other
292 cultivars. However, there was no geographical effect on the number of offspring sired by
293 males, which is consistent with the observation that these males produced the same amount of

294 spermatophore during their lifetimes. The interaction term between male mating numbers and
295 male larval nutrition indicate that benefits of multiple copulations for a given male depended
296 on his larval nutrition (Fig. 2). For example, males from Carignan obtained more offspring
297 due to multiple matings during their life in comparison with males from Mourvèdre (Fig. 2).

298 *Male reproductive investment over successive matings*

299 For each mating opportunity, the mating success of males was relatively high (range 72.7–
300 100%), and was not affected by male larval origin or mating history. The volume of
301 spermatophore transferred to the female at each mating (from the first to the fifth mating) was
302 affected by the male mating history (Fig. 3a; LR = 1548.74, $P < 0.0001$) and the male larval
303 origin (Fig. 3a; LR = 43.07, $P < 0.0001$). It was positively correlated with male pupal mass
304 (LR = 26.20, $P < 0.0001$) but not with female pupal mass (LR = 1.85, $P = 0.174$). The
305 spermatophore produced by males at their first mating was 3–5 times larger than
306 spermatophores transferred during subsequent matings, irrespective of the male larval origin
307 (Fig. 3a). At a given site, the males from Carignan produced significantly smaller
308 spermatophores than males from the two others cultivars, but there was no geographical effect
309 on the spermatophore volume produced by males from Grenache among the three
310 geographically distinct sites. The numbers of eupyrene and apyrene sperm produced by males
311 were also affected by male mating history (Fig. 3b and 3c, respectively; eupyrene sperm: LR
312 = 76.44, $P < 0.0001$; apyrene sperm: LR = 105.94, $P < 0.0001$). However, the numbers of
313 eupyrene or apyrene sperm were not influenced by male larval origin (eupyrene sperm: LR =
314 5.48, $P = 0.242$; apyrene sperm: LR = 2.08, $P = 0.721$) or by male pupal mass (eupyrene
315 sperm: LR = 0.42, $P = 0.517$; apyrene sperm: LR = 0.68, $P = 0.410$). Males transferred more
316 eupyrene and apyrene sperm during their first mating compared with their subsequent
317 matings, and also transferred more sperm during their second mating compared with their
318 fifth mating (Fig. 3b and 3c).

319 *Consequences for the reproductive output of females*

320 The number of eggs laid by a female at each mating strongly depended on the male mating
321 history (Fig. 4a; LR = 99.53, $P < 0.0001$) and larval origin (LR = 47.74.65, $P < 0.0001$). It
322 was also positively correlated with the female pupal mass (Fig 4a; LR = 34.65, $P < 0.0001$)
323 but not the male pupal mass (LR = 2.76, $P = 0.097$). At Perpignan, females mated to males
324 from Mourvèdre laid significantly fewer eggs than females mated to males from the other two
325 cultivars, but there was no geographical effect on the fecundity of females mated with males
326 from Grenache at the three geographically distinct sites. Moreover, females that had copulated
327 with virgin males (first mating) had a higher level of fecundity than females mated to non-
328 virgin males (subsequent matings), and females mated to males that had mated four times
329 previously laid fewer eggs than females mated with males that had mated once or twice (Fig.
330 4a). Female fertility depended on the male larval origin (Fig. 4b; LR = 35.38, $P < 0.0001$) and
331 was positively correlated with female pupal mass (LR = 7.77, $P = 0.005$) but not with male
332 pupal mass (LR = 2.71, $P = 0.099$). Females mated with males from Mourvèdre had fewer
333 hatched eggs than females mated with males from Carignan or Grenache. However, female
334 fertility did not depend on male mating history (Fig. 4b; LR = 1.74, $P = 0.783$), suggesting
335 that males provide female with sufficient sperm to fertilize the same proportion of eggs over
336 five successive matings.

337 *Male 'quality' and motivation to mate in both sexes*

338 The latency period prior to mating was affected by male mating history (LR = 28.50, $P <$
339 0.0001) but not by male larval origin, or male or female pupal mass (LR = 5.06, $P = 0.281$,
340 LR = 0.16, $P = 0.689$, and LR = 0.82, $P = 0.366$, respectively). Male matings occurred sooner
341 for the first mating (13.0 min, ranged from 10.8 to 15.0 min according to the larval origin)
342 relative to the successive matings (mean for the 2nd, 3rd, 4th and 5th mating: 15.5 min, ranged
343 from 9.3 to 20.4 min according to the larval origin).

344 Male activity (expressed as the proportion of time a male spent in activity divided by the
345 latency period prior to mating) was not influenced by male larval origin (LR = 4.81, $P =$
346 0.308). Regardless of their larval origin, males spent on average between 41.9% (males from
347 Mourvèdre) and 55.5 % (males from Grenache) of their time in courtship. Moreover, the time
348 spent in courtship was not dependent on male mating history (LR = 4.19, $P = 0.381$), ranging
349 from 37.4% (5th mating) to 50.3% (2nd mating) of male courtship activity.

350 However, female motivation to mate (i.e. the proportion of time spent calling expressed as the
351 time spent calling divided by the timing of onset of mating) was affected by the male larval
352 origin (Fig. 5; LR = 39.85, $P < 0.0001$) and male mating history (Fig. 5; LR = 17.47, $P =$
353 0.002). Females were more motivated to mate with virgin males or males that had mated once
354 compared with males that had mated 4 or 5 times (Fig. 5). At a given site, females in the
355 presence of males from Mourvèdre were less motivated to mate (calling from 18 to 24% of
356 the time according to male mating history) than females mated with males from Carignan
357 (calling from 22 to 50% of the time depending on male mating history). Among the sites,
358 females paired with males from Grenache in Perpignan spent less time in the calling position
359 than females paired with males from Grenache in Estézargues or Sénas.

360

361 **DISCUSSION**

362 We found that the lifetime reproductive output of males was closely linked to their larval
363 nutrition. Indeed, male larval nutrition on the different grape cultivars affected male
364 longevity, male mating capacity and therefore, the number of offspring sired by males over
365 their lifetime. As expected, male reproductive investment decreased over successive matings,
366 and was largely affected by male larval nutrition on the different grape cultivars and among
367 geographically distinct sites. The male spermatophore volume and the number of sperm in

368 each ejaculate decreased from the first to subsequent matings, and these parameters were
369 affected by the cultivar on which the male larvae were reared. These factors had major
370 repercussions for female reproductive output. Females mated with males producing the largest
371 spermatophore and more sperm (e.g. males from Grenache) had greater fecundity and fertility
372 than females mated with males producing small spermatophores and less sperm (e.g. males
373 from Mourvèdre) across different mating ranks and females were less motivated to mate with
374 the ‘lower quality’ males from Mourvèdre. Moreover, females mated with virgin males (i.e.
375 their first mating) had a greater fecundity than females mated with non-virgin males (i.e. their
376 subsequent matings), regardless of the male larval origin. Thus, females were more motivated
377 to mate with virgin males having high spermatophore quality than with non-virgin males,
378 which transferred less nutritive substances and fewer sperm at mating. Our results suggest that
379 ‘male quality’ depended on both male larval origin and mating history, and had major
380 consequences for female reproductive output. Moreover, females were able to discriminate
381 among these males, and to receive large direct benefits were more motivated to mate with
382 males having high sperm quantity (virgin males or males from certain cultivars or
383 geographical locations).

384 *Larval nutrition, male mating capacity and lifetime reproductive investment*

385 For each mating opportunity, the mating success (i.e. the probability of a male acquiring a
386 mate) of males did not vary according to their larval origin, and remained high and constant
387 over successive matings [min: 80%; max: 100%], suggesting that almost all males had a non-
388 limited mating capacity until their death (based on an inter-mating recovery period of 24 h).
389 However, their larval origin and pupal mass strongly influenced male longevity, with small
390 males reared on Carignan in Perpignan living for a shorter period than large males from
391 Grenache; consequently, these males had fewer mating opportunities over their lifetime *in*
392 *natura*. This may be partly related to the pupal mass because smaller males have less energy

393 reserves than larger males (Muller et al., unpublished data), and cannot afford to invest in
394 both somatic maintenance and reproductive effort (Boggs 2009, Boggs & Freeman 2005).
395 Indeed, males in good condition may be better competitors and have generally better mating
396 success than males in poor condition, without incurring any survival cost (Engqvist 2011,
397 Grandison et al. 2009). Indeed, these males had more energy reserves to invest in somatic
398 maintenance and/or reproduction and are expected to outcompete smaller males reared on less
399 nutritive host plants. We found that the number of male matings was positively related to
400 male longevity: the longer a male lived, the better its chance of reproducing several times
401 (Molleman et al. 2009). Our results suggest that males from Grenache in Estézargues and
402 Sénas were in the best condition, investing in both somatic maintenance (lived for > 8 days)
403 and reproduction (had the largest number of copulations).

404 *Male quality: a combined effect of larval nutrition and mating history*

405 Male reproductive investment, besides to depend on the male larval nutrition, markedly
406 decreased with increasing number of copulations, which was largely because of male inability
407 to replenish resources in the adult stage. Between the first and subsequent matings of males,
408 there was a > 60% decrease in spermatophore volume, confirming that male spermatophore
409 production is very costly (Vahed 1998). Thus, in *L. botrana* and more generally in capital
410 breeder species, males have only a single nutrient-rich spermatophore, which is produced
411 using energy reserves derived from larval nutrition. We previously demonstrated in a
412 laboratory strain of *L. botrana* species that the first spermatophore of males plays a crucial
413 role in female egg production (Muller et al. 2016), and the present study confirms this finding
414 in wild populations of *L. botrana*. This is consistent with the general assumption that male
415 multiple mating can result in the depletion of specific ejaculate components, resulting in
416 decreased fecundity and fertility of their mates (Pérez-Staples et al. 2008, Wigby et al. 2009).
417 However, our results also indicate that the second spermatophore delivered by *L. botrana*

418 males was 66–80 % smaller than the first (according to their larval origin) but the fecundity of
419 the female mated to a once-mated male decreased by only 5–40% according to male larval
420 origin. This suggests that spermatophore size may not be a reliable predictor of female
421 fecundity, and that the quality of the spermatophore rather than its quantity might better
422 explain the variation observed in female fecundity (Bissondath & Wiklund 1996, Muller et al.
423 2015). Both the number of sperm and/or the composition of the ejaculate (e.g. accessory gland
424 secretions) can affect female fecundity (reviewed by Perry et al. 2013), and we recently found
425 that protein-derived spermatophores are a key factor in female reproductive output (Muller et
426 al., unpublished data).

427 The numbers of apyrene and eupyrene spermatozooids also decreased with increasing number
428 of matings. Eupyrene and apyrene spermatogenesis is known to occur at different stages
429 during moth development (Friedländer et al. 2005). Eupyrene spermatogenesis typically
430 begins during the later larval instar stages and ceases at pupation, while apyrene
431 spermatogenesis usually starts just prior to pupation and continues throughout adulthood. All
432 the *L. botrana* males sampled in this study emerged with a finite number of eupyrene sperm,
433 and the males did not release all sperm during the first mating, but retained some for future
434 mating opportunities, ensuring fertilization of the same proportion of female eggs over
435 consecutive matings. However, female fertility was affected by male larval origin, with
436 females mated with males from Mourvèdre having reduced fertility compared with females
437 mated with males from Grenache or Carignan. Males from Mourvèdre were likely to have low
438 quality sperm, and although they transferred the same number of eupyrene sperm as males
439 from the other cultivars, they were not able to fertilize > 70% of female oocytes, regardless of
440 their mating history. As with the spermatophore volume, the quantity of sperm (which is
441 always in excess compared with the number of eggs) is probably a minor factor relative to its
442 quality (Snook 2005, Werner & Simmons 2008). Numerous sperm traits that contribute to

443 paternity (including sperm size, viability, and mobility) are known to influence fertilization
444 efficiency in moths (Morrow & Gage 2000, Perry et al. 2013). In *L. botrana*, male larval food
445 composition could directly influenced sperm quality, as demonstrated in other moth species
446 (Gage & Cook 1994, Cordes et al. 2015).

447 *Implications for the evolution of female mate choice*

448 Because ‘male quality’ depends on both the male larval nutrition and male mating history,
449 females should be able to distinguish between males of different qualities on the basis of these
450 two factors. This study provides initial evidences that females seem to prefer to mate (1) with
451 males originated from cultivars that enhance their reproductive performances and; (2) with
452 virgin males rather than already mated males.

453 Firstly, the female motivation varied with male origin, with females being less motivated
454 (spent less time in the calling position) to mate with males having lower spermatophore
455 quality (those reared on Mourvèdre) than males from the other cultivars, suggesting that the
456 females used cues (perhaps chemical fingerprints of males having different host origins) that
457 provided information on male condition (Costanzo & Monteiro 2007, Harris & Moore 2005),
458 and therefore spermatophore quality.

459 Secondly, the latency period to mating significantly increased with increasing number of
460 matings, with non-virgin males that had already mated taking 20–25% more time to mate than
461 virgin males. This suggests that after their first mating, males needed more time to
462 successfully mate. This may be because of cumulative fatigue resulting from successive
463 mating, or because the females were able to detect that these males were potentially sperm-
464 depleted, and were more reluctant to mate with them. Analysis of the precopulatory behaviors
465 of each sex suggested that the amount of time required to mate by experienced males was not
466 merely a result of cumulative fatigue, because males were equally active during courtship

467 regardless of their mating history. Thus, the longer latency period prior to mating for non-
468 virgin males was probably the result of female reluctance to mate with previously mated
469 males. Indeed, females were more motivated to mate with virgin males than with non-virgin
470 males. In a recent study of *L. botrana* involving mate-choice experiments, we demonstrated a
471 female preference for virgin males, which maximized the direct benefits associated with
472 receiving large spermatophores (Muller et al. 2016).

473 Nevertheless, this study was a laboratory experiment and there is no yet demonstrated
474 evidence of the existence of a female mate choice *in natura* in this species. Models usually
475 predict that the level of female choosiness should depend on the importance of the cost of
476 searching mates (ie, the proportion of lifetime devoted to searching for mates) which depends
477 on the operational sex ratio and the encounter rate (Bleu et al. 2011, Etienne et al. 2014).
478 Highly choosy females run the risk of remaining unmated and the level of choosiness is likely
479 to reach a value that counterbalances the benefits of obtaining high quality males and the costs
480 of mating and of remaining unmated (Kokko & Mappes 2005). Typically, females should
481 mate fairly indiscriminately when they first mate because of the large fitness cost of not
482 mating (Worthington & Kelly 2016). However, in *L. botrana*, the occurrence of mating
483 failures seems to be low (Torres-Vila et al. 2004) and our previous mate-choice study (Muller
484 et al. 2016) indicates that virgin females have evolved the capacity to discriminate among
485 males based on male mating experience.

486 *Conclusion and future perspectives*

487 Our results highlight the overall importance of larval nutrition in male mating capacity and
488 lifetime reproductive investment, all of which could modulate the reproductive strategies of
489 this pest. In *L. botrana* species, both sexes are expected to be choosy about their mating
490 partners because both males and females greatly vary in their reproductive quality according
491 to intrinsic and extrinsic conditions (Moreau et al. 2006, Muller et al. 2015). Indeed, males

492 and females have non-trivial reproductive costs (Harari et al. 2011). Firstly, females that
493 invest many limited resources in egg production would be expected to preferentially mate
494 with high quality males (virgin males or males in good condition as a consequence of their
495 larval nutrition) to obtain large direct benefits from mating (such as large and nutrient-rich
496 spermatophores). Secondly, males are also expected to exhibit some mate choice, because
497 spermatophore production is costly and males only produce one nutrient-rich spermatophore
498 throughout their lifetime (Bonduriansky 2001). Moreover, in this moth species, both sexes
499 invest in mate-finding traits; *L. botrana* females emit costly pheromones to attract mates
500 (Harari et al. 2011, Umbers et al. 2015) and males actively search mates by following these
501 chemical signals. The occurrence of mate choice by both sexes of this species should be
502 further investigated.

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661 Figure 1: Percentage of *L. botrana* males having N mating events (from 1 to > 5) with a
662 different female each day during their lifetime, as a function of male origin (cultivar and site).
663 Numbers inside bars are the sample sizes.

664

665 Figure 2. Mean \pm SEM of (a) the spermatophore volume, (b) the number of eupyrene sperm,
666 and (c) the number of apyrene sperm for *L. botrana* males of different larval origin (cultivar
667 and site), as a function of their mating history (the first to the fifth mating). Black squares and
668 dashed line: Carignan in Perpignan; black downward triangles and full line: Grenache in
669 Perpignan; black upward triangles and dotted line: Mourvèdre in Perpignan; white circles and
670 full line: Grenache in Sénas; and white diamonds and full lines: Grenache in Estézargues

671

672 Figure 3. Mean \pm SEM of the motivation to mate for *L. botrana* females mated with males of
673 different larval origin (cultivar and site), as a function of their mating history (the first to the
674 fifth mating). Symbols and lines as for Figure 2.

675

676 Figure 4. Lifetime number of offspring sired by *L. botrana* males, as a function of the number
677 of male matings. Symbols and lines as for Figure 2.

678

679 Figure 5. Mean \pm SEM of (a) the female fecundity (number of eggs laid) and (b) the female
680 fertility (percent of eggs hatched) mated with males of different larval origin (cultivar and
681 site) and males having different mating histories (the first to the fifth mating). Symbols and
682 lines as for Figure 2.

Table 1. Traits of *L. botrana* males having different larval origins (cultivar and site). The capital letters in brackets correspond to the various sites: (P) Perpignan, (S) S nas, and (E) Est zargues. In each column, values having different uppercase letters are significantly different ($P < 0.05$).

Cultivar	Male pupal mass (mg)	Male longevity (days)	Male number of matings	Male lifetime reproductive investment		
				Total spermatophore quantity ($\text{mm}^3 \cdot 10^{-6}$)	Total number of eupyrene sperm	Total number of apyrene sperm
Carignan (P)	5.1 ^a [4.9 ; 5.3]	6.0 ^a [5.5 ; 6.5]	3.3 [2.8 ; 3.8]	96.6 ^a [80.87 ; 116.3]	4665 [4821 ; 7829]	72467 [50988 ; 108750]
Mourv�dre (P)	5.5 ^b [5.4 ; 5.7]	7.6 ^b [7.0 ; 8.2]	4.3 [3.7 ; 4.9]	125.0 ^a [111.3 ; 139.9]	6118 [4877 ; 7322]	91609 [74296 ; 108750]
Grenache (P)	5.3 ^{ab} [5.0 ; 5.5]	7.3 ^b [6.6 ; 8.0]	3.9 [3.3 ; 4.6]	132.7 ^b [111.5 ; 155.1]	6315 [4877 ; 7829]	75497 [57205 ; 93589]
Grenache (E)	6.2 ^c [5.9 ; 6.4]	8.2 ^b [7.6 ; 8.8]	4.9 [4.2 ; 5.6]	177.2 ^b [145.0 ; 212.9]	8363 [6763 ; 10112]	80340 [65881 ; 96104]
Grenache (S)	6.2 ^c [6.0 ; 6.5]	8.6 ^b [8.0 ; 9.1]	5.4 [4.8 ; 6.1]	197.8 ^b [168.5 ; 226.5]	7950 [6371 ; 9571]	87093 [73264 ; 101928]

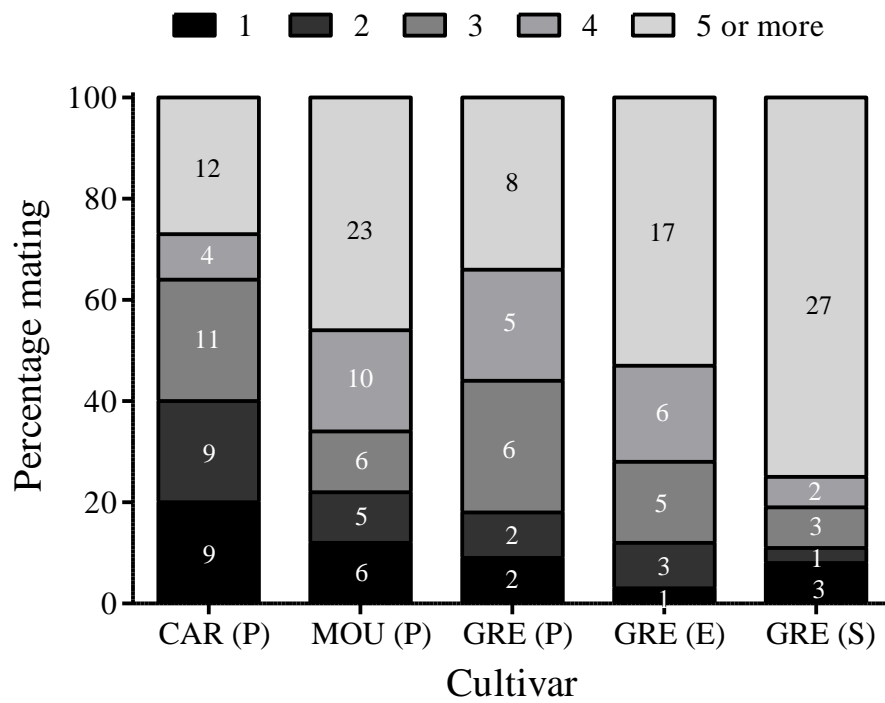


Figure 1

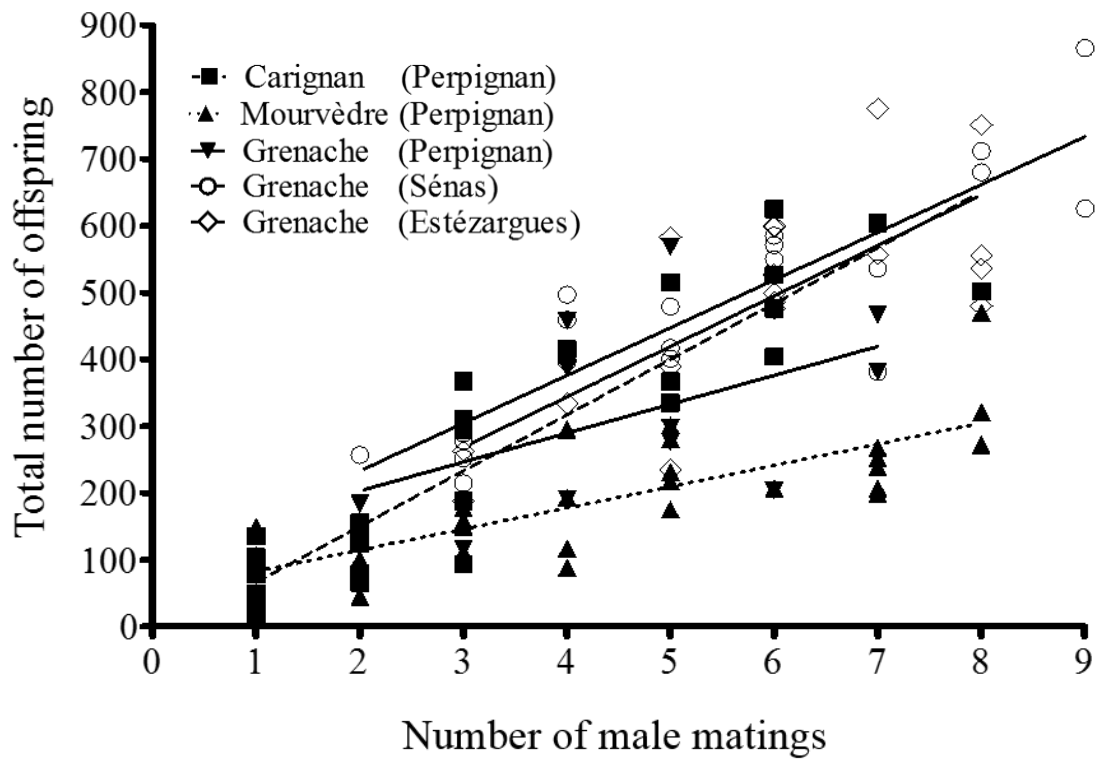


Figure 2

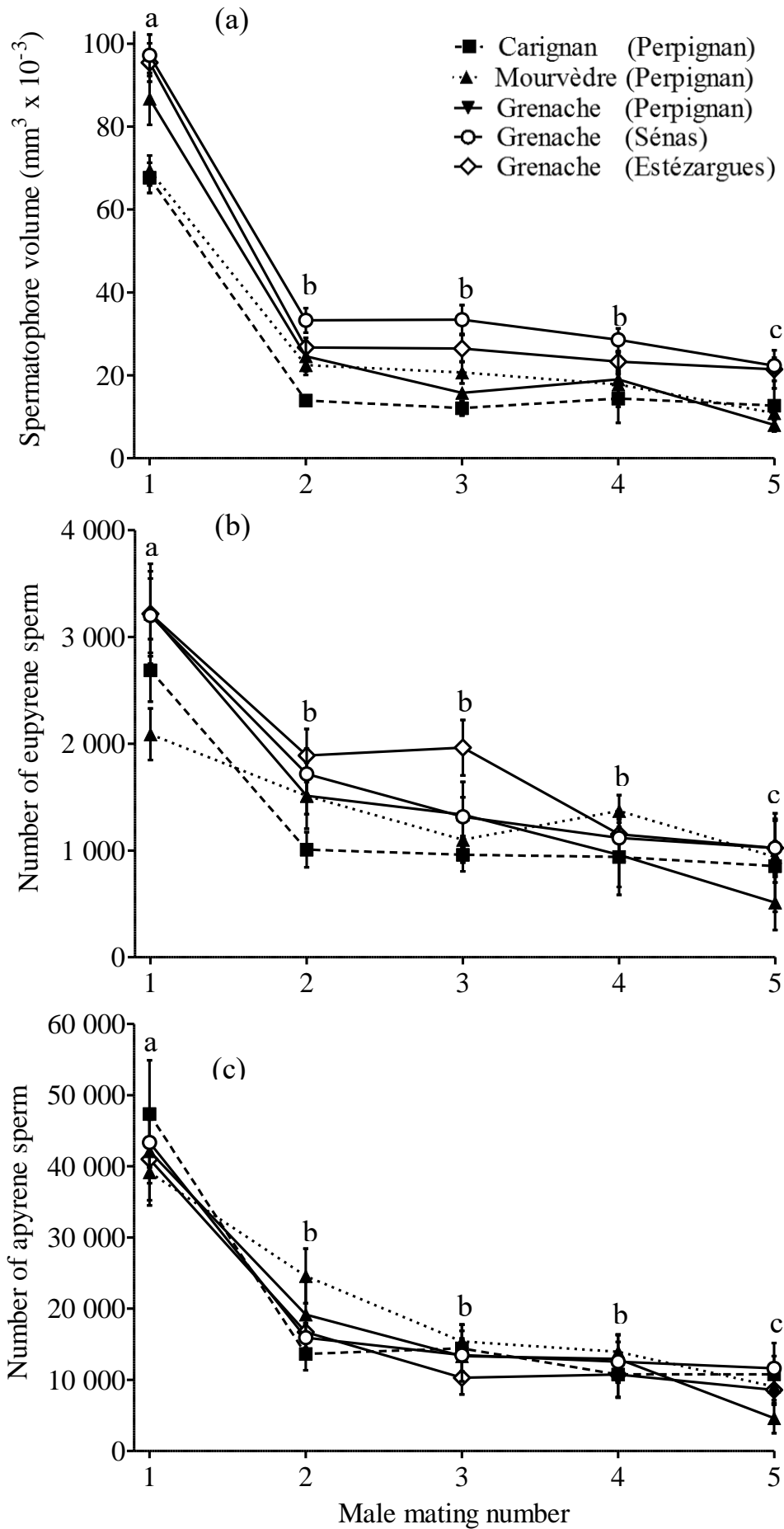


Figure 3.

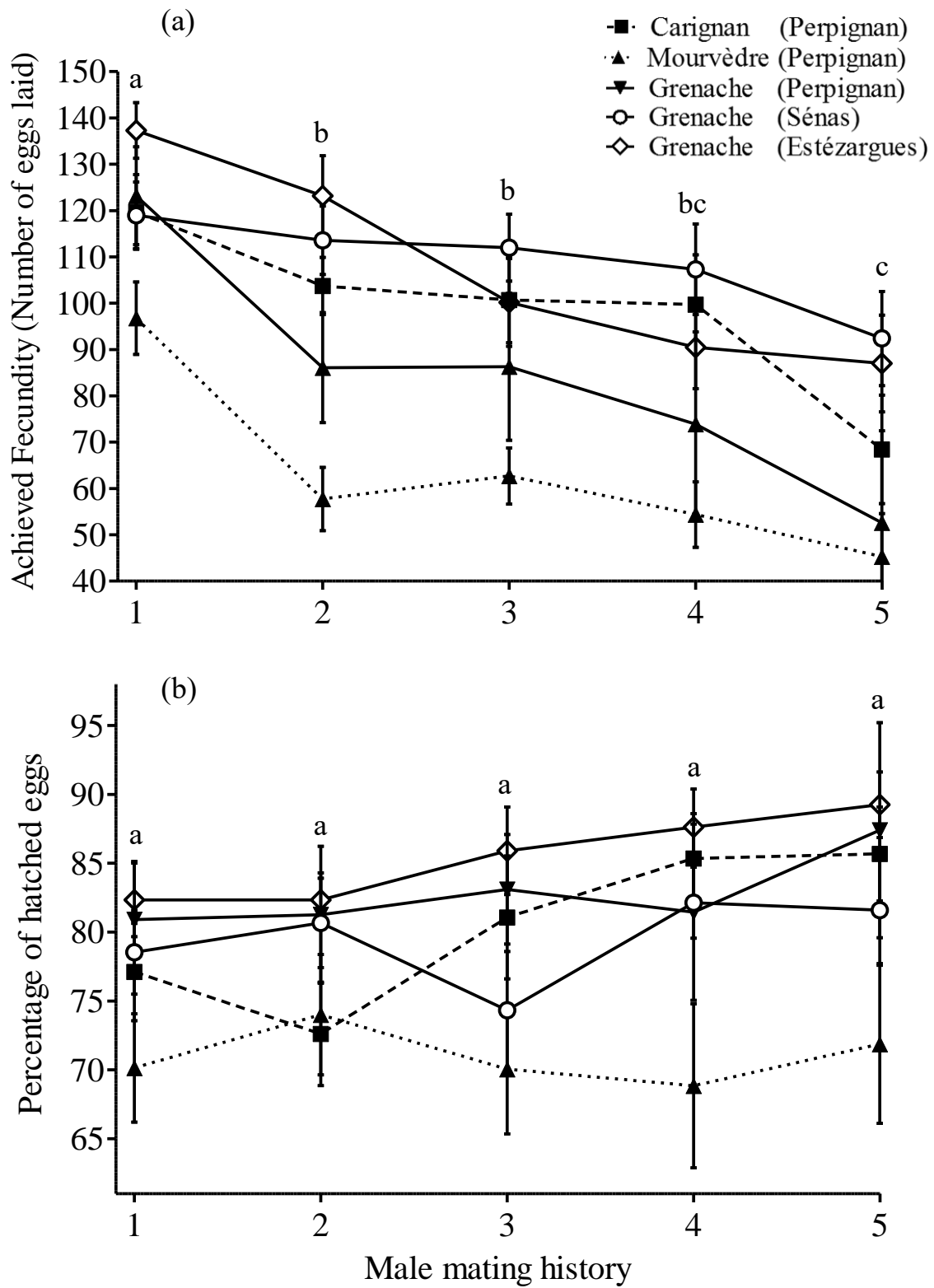


Figure 4.

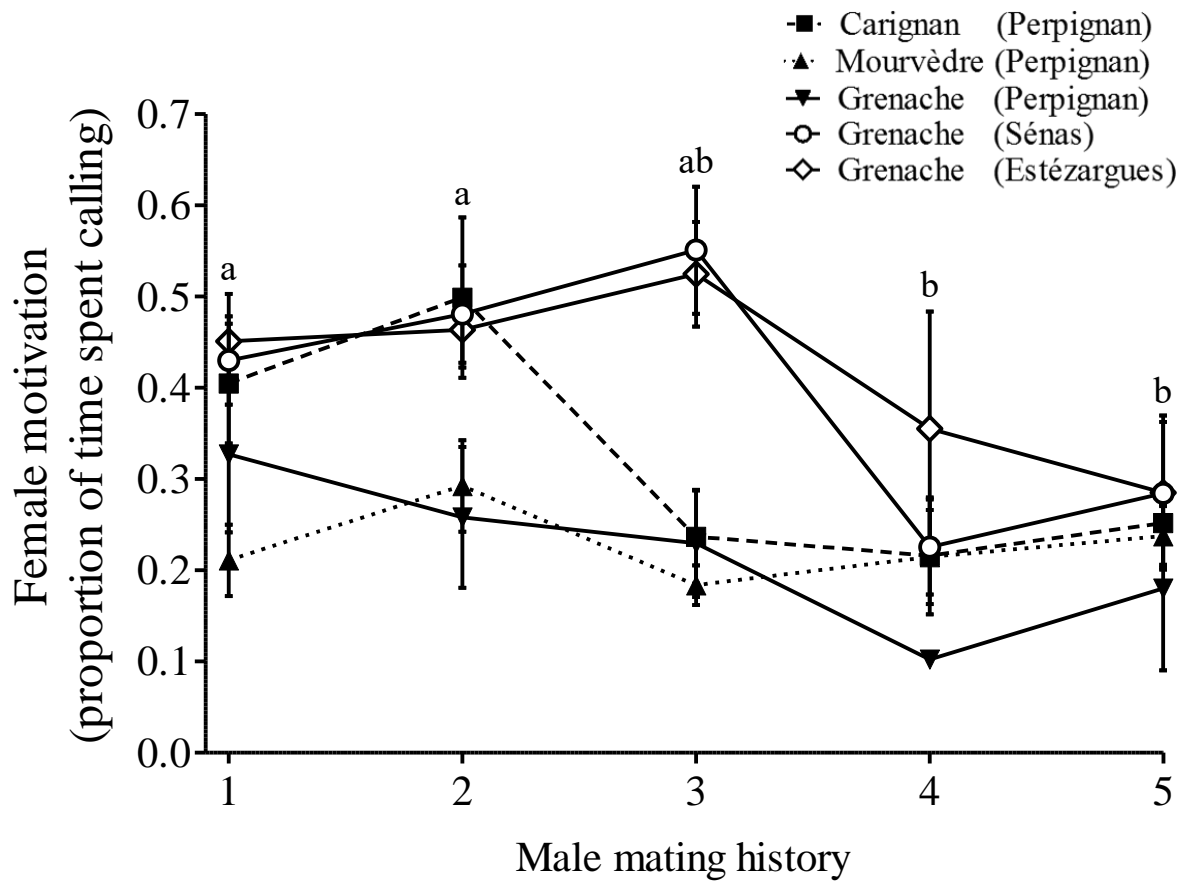


Figure 5.