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

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


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

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

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

For species in which males provide females with material resources including a nutritive ejaculate (for example, spermatophores in some lepidopteran species), the influence of male mating frequency on future reproductive output can also be extremely pronounced (Torres-Vila & Jennions, 2005; Wedell et al. 2002). Because ejaculate production is costly (Dewsbury, 1982), male performance usually declines across multiple matings, leading to diminishing reproductive returns for males (reviewed by Simmons, 2001). Moreover, males may be limited in the amount of sperm they can transfer to a female during mating (Marcotte, Delisle, & McNeil, 2005), and male mating history (the number of previous matings) is certainly a key factor determining female fitness, especially in species in which males can keep copulating despite being sperm depleted (Damiens & Boivin, 2006; Steiner, Henrich, & Ruther, 2008). It has been commonly assumed that males have to face trade-offs between investment in somatic maintenance and investment in reproduction because they have finite resources to invest (Barnes & Partridge, 2003; Stearns, 1992). Such trade-offs typically arise under food limitation, because male expenditure in ejaculate production is constrained in part by resource availability; consequently, males have to invest in either current or future reproduction (Simmons, 2001). In capital breeders, which rely mainly on larval reserves for successful reproduction, the resources needed to produce a nutritive ejaculate can be a limiting factor. Therefore, ejaculate production could be related to the number of copulations and male larval nutrition, but few studies have reported the quantitative and qualitative relationships involved. Diet quality can have a significant influence on the rate at which males produce ejaculate, the quality of the seminal fluid proteins and the effectiveness of the ejaculate in achieving fertilization (Arnqvist & Danielsson, 1999; Gage & Cook, 1994; Simmons & Kvarnemo, 1997). When males lack adequate protein sources or when they have developed on nutritionally limited host plants, critical depletion of their ejaculate generally occurs during successive matings (Gage & Cook, 1994). However, most studies have focused on the factors affecting male reproductive output following emergence, particularly during the first two mating events (Cordes et al. 2015; Delisle & Hardy, 1997; Tigreros, 2013) but not on the trade-off between larval nutrition and the male's entire lifetime reproductive investment.

In this context, our study goals were to assess whether (1) larval nutrition is important for male mating capacity and lifetime reproductive investment, (2) male larval nutrition and male mating history together affect males' quality as mates and (3) females prefer to mate with 'high-quality mates' in order to obtain larger direct benefits. To answer these questions, we used the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae), which is a very important pest of grapes worldwide. Several studies of this species have already shown marked effects of larval nutrition on male and female fitness (Moreau, Benrey, & Thiéry, 2006; Moreau, Thiéry, Troussard, & Benrey, 2007; Muller, Thiéry, Moret, & Moreau, 2015). However, the lifetime reproductive capacity of male moths of this species remains unknown because most studies have concerned only the first mating of individuals (Moreau et al. 2006, 2007; Muller et al. 2015; see Torres-Vila, Rodriguez-Molina, Roehrich, & Stockel, 1999 for an exception). In the present study, we provided wild *L. botrana* males that developed on different grape cultivars and in different vineyards with unlimited access to females and investigated the lifetime reproductive success of the males. We also investigated the consequences for the reproductive output of females as a function of male larval origin and mating history. In a first step we explored variation in male reproductive investment (spermatophore size, number of sperm) during successive matings. We predicted that (1) male reproductive investment and mating capacity would be affected by male larval nutrition on different cultivars and (2) male quality would depend on both their larval nutrition and mating history. In a second step, we studied the consequences of male larval nutrition and mating history on the reproductive output of females (fecundity and fertility). We predicted (3) that female fitness would be affected by both male larval origin and mating history and (4) that females would be more motivated to mate with males of 'high quality', thus receiving larger nutrient-rich spermatophores.

## METHODS

### *Field Sampling*

*Lobesia botrana* is a major pest of grapes. It is widely distributed in most European vineyards and is now present in the U.S.A., where three or four larval generations occur each year, depending on latitude. First-generation larvae of *L. botrana* were collected in the field during June 2013. To test for a cultivar effect within a given population, larvae were sampled from three grape, *Vitis vinifera*, cultivars ('Carignan', 'Mourvèdre' and 'Grenache') in the same vineyard (Perpignan, France; 42°44′7.063″N, 2°52′56.441″E), ensuring the same abiotic conditions (temperature, light exposure, humidity) for larval development. The three chosen grape varieties are biochemically very different, especially in their phenolic contents (Teissedre & Chervin, 2011). Indeed, 'Carignan' and 'Grenache' grape extracts contain less total phenols than Mourvèdre grape extracts (Jensen, Demiray, Egebo, & Meyer, 2008). To test for a geographical effect, we sampled larvae from the cultivar 'Grenache' from two additional geographically distinct French vineyards: Estézargues (43°56′49.781″N, 4°39′39.372″E) and Sénas (43°43′54.251″N, 5°1′45.621″E). Larvae were sampled at the end of the larval cycle (fifth instar), following construction of glomerulae made of flower buds aggregated in larval silk (phenology 17–25; Eichhorn & Lorenz, 1977). Larvae usually complete their development in a single grape bunch, and each glomerulus is only occupied by a single larva (Torres-Vila, Stockel, & Rodriguez-Molina, 1997). To collect newly emerged adults, larvae at the end of their development were placed in large polyethylene boxes (60 × 40 cm and 21 cm high) in the laboratory and fed ad libitum on grape bunches from the same cultivar and site where they developed, and were incubated at 22 ± 1 °C, 60 ± 10% relative humidity, and under natural photoperiod conditions. The larvae were checked daily until pupation at which time they were gently removed from their glomerulae. The pupae were weighed to the nearest 0.1 mg using a Precisa 262 SMA-FR microbalance, placed individually in glass tubes (70 × 9 mm diameter) stoppered with cotton wool plugs, and

stored at 22 ± 1 °C under natural photoperiod conditions. The pupae were checked each morning, and newly emerged adults were visually sexed by examination of the ventral tip of the abdomen.

To assess the importance of male larval origin and mating history on male reproductive investment and female reproductive output, 2-day-old males of different larval origin (cultivar and site) were given daily mating access to a new 1- or 2-day-old standardized virgin female; this was continued until death of the male. The standardized females came from an inbred strain (INRA Bordeaux) maintained without diapause on a semi-artificial diet. The use of this inbred strain helps to minimize genetic variation between females and allows us to detect the male's effect on female reproduction (see Moreau et al. 2007; Muller et al. 2015 for more details). Males used for the following mating experiments were randomly distributed into two subsamples. The first subsample was used to evaluate the effect of male origin (cultivar and site) and mating history on male lifetime reproductive investment, and was also used to monitor the male precopulatory behaviour for each mating event. The second subsample was used to assess the consequences of male origin (cultivar and site) and mating history on the reproductive output of females.

### *Ethical Note*

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

### *Mating Procedure*

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

### *First Subsample: Precopulatory Behaviours of Males and Females*

For the first subsample the pair's sexual activity was videotaped (Sony HDR CX220E) until mating; only recordings of successful matings (with effective spermatophore transfer or female egg laying) were analysed. The latency period prior to mating (the time elapsed from male/female pairing until coupling) was recorded along with the occurrence of behaviours reflecting female and male sexual motivation (as described by Muller et al. 2015). The latency to mate is a first measure that accurately reflects the reluctance or acceptance to mate in no-choice tests (Edward, 2014; Muller, Arenas, Thiéry, & Moreau, 2016; Muller, Teixeira-Brandao, Thiéry, & Moreau, 2016). Moreover, in *L. botrana* a female that is ready to mate signals readiness by releasing sex pheromone at dusk, which is an action that represents a fitness cost (Harari, Zahavi, & Thiéry, 2011). To do this the female assumes a calling position with wings raised and the pheromone gland exposed. This behaviour reflects the tendency for a female to mate, and therefore we used it as a proxy of female motivation. Thus, we recorded data on the female's motivation to mate (expressed as the time a female spent calling divided by the courtship duration ×100). To evaluate mating ability and sexual vigour of males in courtship, we also recorded data on the percentage of male activity (the time spent in movement by the male expressed as a percentage of the total courtship period).

### *First Subsample: Male Reproductive Performance*

Immediately following mating the females were chilled (−25 °C for 10 min), then dissected on a glass side. The bursa copulatrix containing the male spermatophore was removed and measured. The spermatophore produced by *L. botrana* males is very small (<1 mg) and consequently difficult to weigh accurately. We estimated the spermatophore size by extrapolating its volume; this is a well-established method used for small moths including *L. botrana* (Muller et al. 2015; Torres-Vila et al. 1999). The spermatophore length ($l$), width ($w$) and thickness ($t$) were measured using a stereomicroscope (Nikon SMZ1500) at a magnification of 20×, and the spermatophore volume was estimated as an ellipsoid balloon [$V = \pi/6\ (l \times w \times t)$], as described previously (Muller et al. 2015; Torres-Vila et al., 1999). As in all Lepidoptera, male *L. botrana* transfer fertile eupyrene sperm and nonfertile anucleate apyrene sperm at mating. The sperm-containing ampulla was ruptured in a drop of distilled water and the sperm mass was gently stirred to ensure dispersion. In Lepidoptera at this stage the eupyrene sperm are encysted in bundles, and each bundle contains 256 eupyrene sperm (Cook & Gage, 1995). The number of intact bundles was counted at 40×/0.65 magnification using a Nikon Eclipse E600 microscope; this number was multiplied by 256 to estimate the total number of eupyrene sperm. The solution was then washed from the slide into a 1.5 ml centrifuge tube and diluted with distilled water. Four subsamples (10 µl) were removed from the diluted sperm solution, and the apyrene sperm were counted by microscopy (Nikon Eclipse E600; 100× magnification). The total number of apyrene sperm was estimated by multiplying the average sperm count for the four subsamples (coefficient of variation = 12%) by the dilution factor.

We recorded: (1) male longevity; (2) the total number of matings by males during their life span; (3) the lifetime spermatophore quantity produced and the lifetime number of sperm transferred; (4) the number of offspring sired by males during their lifetime; and (5) the spermatophore volume and the number of sperm transferred at each male mating.

### *Second Subsample: Consequences for Female Reproductive Output*

Following mating (see general mating procedure) the females were held in the mating tube and could oviposit freely on the inside surface of the glass tub. Female survival was checked daily, and following death the eggs were incubated for 7 days under the same conditions as for moth maintenance. We recorded several female traits as a function of male larval origin (cultivar or site) and mating history, including (1) female fecundity (the number of eggs laid per female at each mating) and (2) female fertility (the proportion of hatched eggs for each mating).

### *Statistical Analysis*

All statistical tests were performed using R Software version 3.2.0 (R Development Core Team, 2015). For each analysis we report

the full model with nonsignificant interactions deleted, following the approach of Forstmeier and Schielzeth (2011). The effect of male origin (cultivar and site) on male pupal mass was tested using a one-way ANOVA followed by Tukey's test. A Cox regression was applied to assess the influence of male origin and pupal mass on male longevity. Sources of variation in the total number of matings by males during their lifetime, the lifetime quantity of spermatophores produced by males and the number of offspring sired by males during their lifetime were identified using ANCOVAs, with male larval origin as the explanatory variable and the number of male matings and the male and female pupal masses as covariates. Because the sperm were counted and were overdispersed, a generalized linear model with a negative binomial distribution was used to evaluate the effect of male origin on the total number of sperm transferred by males during their lifetime.

We used a general mixed model with male identity as a random effect to assess the combined effects of male mating history and larval origin on precopulatory behaviour, the spermatophore size, the number of eupyrene and apyrene sperm, and the female fecundity and fertility. Male mating history was recorded as a discrete variable (one, two, three, four or five matings); the data for the previous five, six or seven matings were excluded because the sample size was too small for certain male origins (<5 individuals). Pearson's chi-square tests were used to assess the mating success of males (percentage of successful matings) as a function of larval origin (cultivar and site) and mating history. Because of non-normality, female motivation to mate (percentage of time spent in the calling position) and male activity (percentage of time spent in movement) were arcsine square root transformed prior to analysis. The latency period prior to mating, the male's activity and the female's motivation to mate were analysed using general linear mixed models. Because data on sperm were best approximated by an overdispersed Poisson distribution, we fitted the model with a negative binomial error structure and used the glmmADMB library to perform the analysis, which included male mating history and male larval origin as fixed effects, male and female mass as covariates and male identity as a random factor. The proportion of eggs hatched was analysed using the glmmPQL function with a quasi-binomial error structure.

## RESULTS

### *Male Pupal Mass and Longevity*

Male pupal mass was affected by male larval origin (Table 1; $F_{4,181} = 20.63$, $P < 0.0001$). Males from Mourvèdre in the Perpignan vineyard were larger than those from Carignan (Table 1). Among sites, the males from Grenache in Estézargues and Sénas were heavier than those from this cultivar in Perpignan. Male longevity was also influenced by the origin of the males ($\chi^2_{4,181} = 23.23$, $P < 0.0001$), and was positively related to male pupal mass ($\chi^2_{1,181} = 26.02$, $P < 0.0001$; Table 1). At a given site (Perpignan), the males from Carignan died earlier than males from Grenache and Mourvèdre. However, the longevity of males from Grenache in Perpignan was similar to that of males from Grenache in Estézargues or Sénas. As a consequence of differing longevity, males did not have the same number of mating opportunities over their lifetimes. Consequently, male mating capacity (i.e. the maximum number of matings that males undertook during their lifetimes) was positively related to male longevity ($F_{1,179} = 188.01$, $P < 0.0001$). For example, in a given vineyard the males from Carignan lived an average of 6 days, and tended to mate less often than males from Grenache or Mourvèdre, which lived for more than 7 days (Table 1, Fig. 1). Moreover, among the males from Grenache in Sénas and Estézargues (which lived the longest), 50–75% mated five or more times during their lifetime, while only 30% of the males from Grenache in Perpignan mated at least five times (Table 1, Fig. 1).

### *Male Lifetime Reproductive Investment*

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

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

**Table 1**
Traits of *L. botrana* males with different larval origins (cultivar and site)

| Cultivar | Male pupal mass (mg) | Male longevity (days) | Male number of matings | Male lifetime reproductive investment | | |
|---|---|---|---|---|---|---|
| | | | | Total spermatophore quantity (mm$^3$ × 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] | 72 467 [50 988; 108 750] |
| Mourvèdre (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] | 91 609 [74 296; 108 750] |
| 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] | 75 497 [57 205; 93 589] |
| 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; 10 112] | 80 340 [65 881; 96 104] |
| 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] | 87 093 [73 264; 101 928] |

The capital letters in parentheses correspond to the various sites: (P) Perpignan, (S) Sénas and (E) Estézargues. Numbers in brackets are 95% confidence intervals. In each column, values with different lowercase letters are significantly different ($P < 0.05$).

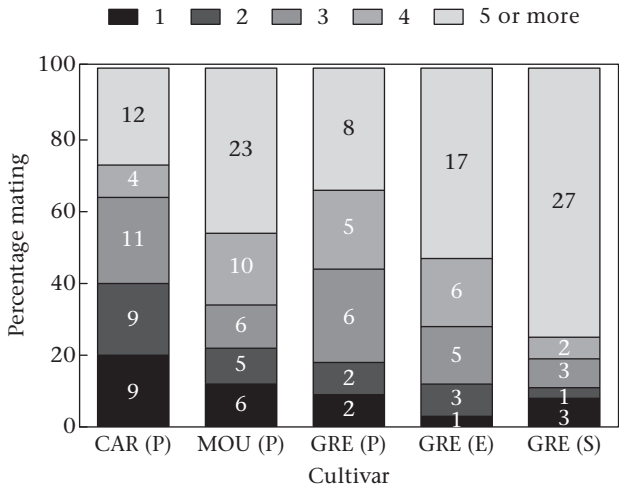


**Figure 1.** Percentage of *L. botrana* males having *N* mating events (from 1 to >5) with a different female each day during their lifetime, as a function of male origin (cultivar and site). Numbers inside bars are the sample sizes.

larval nutrition (Fig. 2). For example, males from Carignan obtained more offspring due to multiple matings during their life in comparison with males from Mourvèdre (Fig. 2).

*Male Reproductive Investment over Successive Matings*

For each mating opportunity, the mating success of males was relatively high (range 72.7–100%), and was not affected by male larval origin or mating history. The volume of spermatophore transferred to the female at each mating (from the first to the fifth mating) was affected by the male's mating history (Fig. 3a; likelihood ratio, LR = 1548.74, $P < 0.0001$) and the male larval origin (Fig. 3a; LR = 43.07, $P < 0.0001$). It was positively related to male pupal mass (LR = 26.20, $P < 0.0001$) but not with female pupal mass (LR = 1.85, $P = 0.174$). The spermatophore produced by males at their first mating was three to five times larger than spermatophores transferred during subsequent matings, irrespective of the male larval origin (Fig. 3a). At a given site, the males from Carignan produced significantly smaller spermatophores than males from the other two cultivars, but there was no geographical effect on the spermatophore volume produced by males from Grenache among the three geographically distinct sites. The numbers of eupyrene and apyrene sperm produced by males were also affected by male

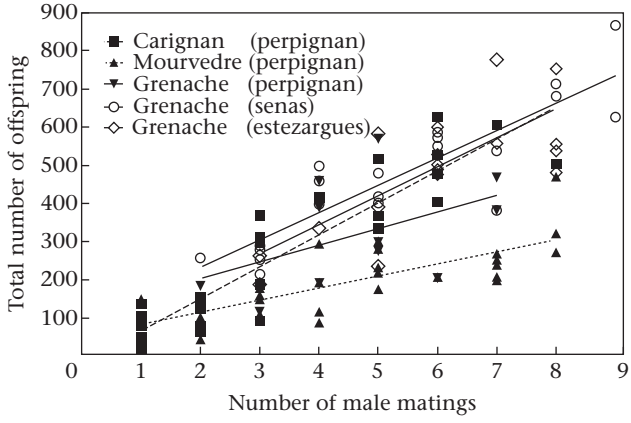


**Figure 2.** Lifetime number of offspring sired by *L. botrana* males of different larval origin (cultivar and site), as a function of the number of male matings.

mating history (Fig. 3b, c, respectively; eupyrene sperm: LR = 76.44, $P < 0.0001$; apyrene sperm: LR = 105.94, $P < 0.0001$). However, the numbers of eupyrene or apyrene sperm were not influenced by male larval origin (eupyrene sperm: LR = 5.48, $P = 0.242$; apyrene sperm: LR = 2.08, $P = 0.721$) or by male pupal mass (eupyrene sperm: LR = 0.42, $P = 0.517$; apyrene sperm: LR = 0.68, $P = 0.410$). Males transferred more eupyrene and apyrene sperm during their first mating than at subsequent matings, and also transferred more sperm during their second mating than their fifth mating (Fig. 3b, c).

*Consequences for the Reproductive Output of Females*

The number of eggs laid by a female at each mating strongly depended on the male's mating history (Fig. 4a; LR = 99.53,

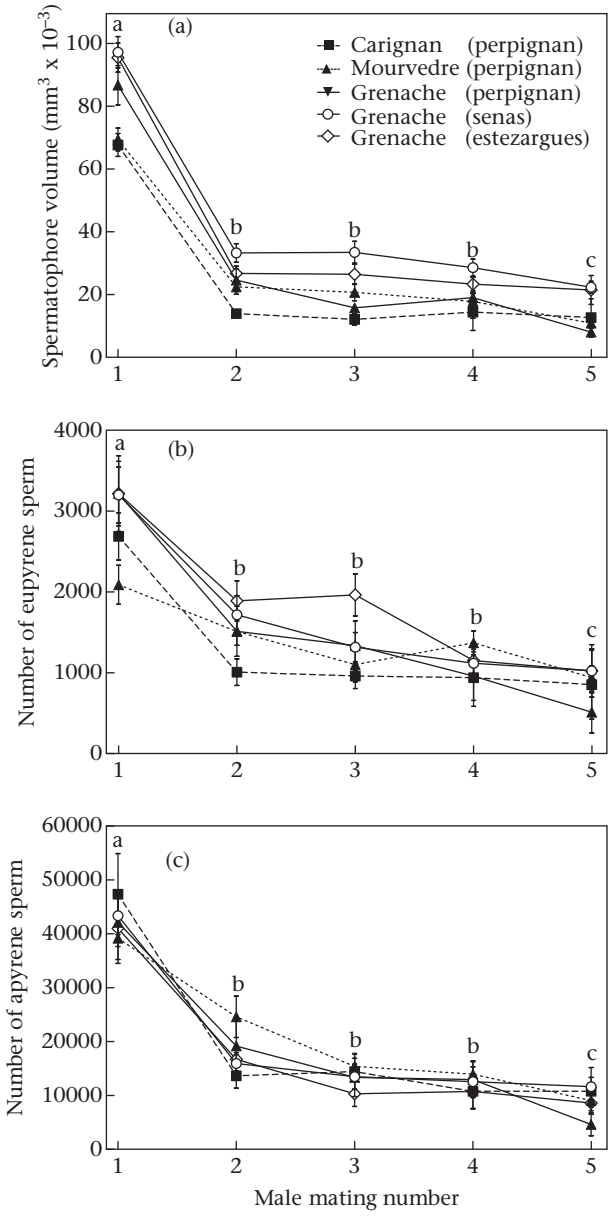


**Figure 3.** Mean ± SEM of (a) the spermatophore volume, (b) the number of eupyrene sperm and (c) the number of apyrene sperm for *L. botrana* males of different larval origin (cultivar and site), as a function of their mating history (the first to the fifth mating).

$P < 0.0001$) and larval origin (LR = 47.74.65, $P < 0.0001$). It was also positively related to the female's pupal mass (Fig. 4a; LR = 34.65, $P < 0.0001$) but not the male's pupal mass (LR = 2.76, $P = 0.097$). At Perpignan, females mated to males from Mourvèdre laid significantly fewer eggs than females mated to males from the other two cultivars, but there was no geographical effect on the fecundity of females mated with males from Grenache at the three geographically distinct sites. Moreover, females that had copulated with virgin males (first mating) had a higher level of fecundity than females mated to nonvirgin males (subsequent matings), and females mated to males that had mated four times previously laid fewer eggs than females mated with males that had mated once or twice (Fig. 4a). Female fertility depended on the male's larval origin (Fig. 4b; LR = 35.38, $P < 0.0001$) and was positively related to female pupal mass (LR = 7.77, $P = 0.005$) but not male pupal mass (LR = 2.71, $P = 0.099$). Females mated with males from Mourvèdre had fewer hatched eggs than females mated with males from Carignan or Grenache. However, female fertility did not depend on the male's mating history (Fig. 4b; LR = 1.74, $P = 0.783$), suggesting that males provide female with sufficient sperm to fertilize the same proportion of eggs over five successive matings.

### *Male 'Quality' and Motivation to Mate in Both Sexes*

The latency period prior to mating was affected by male mating history (LR = 28.50, $P < 0.0001$) but not by male larval origin, or male or female pupal mass (LR = 5.06, $P = 0.281$, LR = 0.16, $P = 0.689$, and LR = 0.82, $P = 0.366$, respectively). Male matings occurred sooner for the first mating (13.0 min, range 10.8–15.0 min according to the larval origin) relative to the successive matings (mean for the second, third, fourth and fifth mating: 15.5 min, range 9.3–20.4 min according to the larval origin).

Male activity (expressed as the proportion of time a male spent in activity divided by the latency period prior to mating) was not influenced by male larval origin (LR = 4.81, $P = 0.308$). Regardless of their larval origin, males spent on average between 41.9% (males from Mourvèdre) and 55.5% (males from Grenache) of their time in courtship. Moreover, the time spent in courtship was not dependent on the male's mating history (LR = 4.19, $P = 0.381$; range 37.4% (fifth mating) to 50.3% (second mating) of male courtship activity).

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

## DISCUSSION

We found that the lifetime reproductive output of males was closely linked to their larval nutrition. Indeed, male larval nutrition on the different grape cultivars affected male longevity, male mating capacity and, therefore, the number of offspring sired by males over their lifetime. As expected, male reproductive investment decreased over successive matings, and was largely affected by male larval nutrition on the different grape cultivars and at geographically distinct sites. The male spermatophore volume and the number of sperm in each ejaculate decreased from the first to subsequent matings, and these parameters were affected by the cultivar on which the male larvae were reared. These factors had major repercussions for female reproductive output. Females mated with males producing the largest spermatophore and more sperm (e.g. males from Grenache) had greater fecundity and

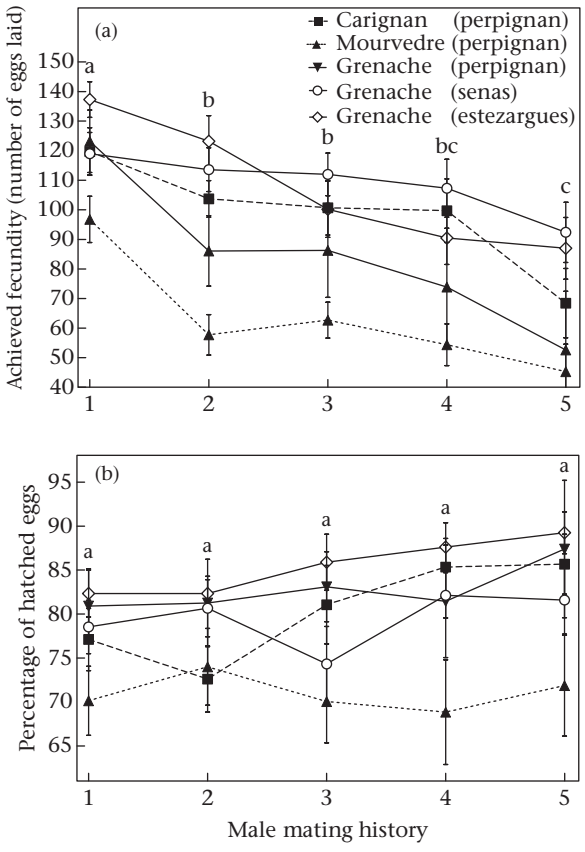


**Figure 4.** Mean ± SEM of (a) the fecundity (number of eggs laid) and (b) the fertility (percentage of eggs hatched) of *L. botrana* females mated with males of different larval origin (cultivar and site) and males with different mating histories (the first to the fifth mating).

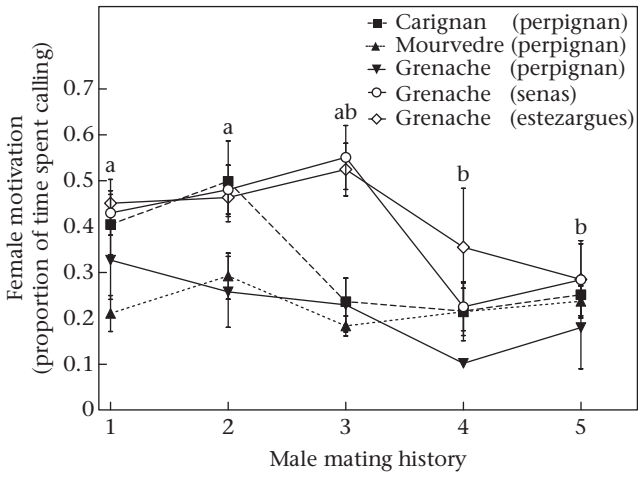


**Figure 5.** Mean ± SEM of the motivation to mate for *L. botrana* females mated with males of different larval origin (cultivar and site), as a function of their mating history (the first to the fifth mating).

fertility than females mated with males producing small spermatophores and less sperm (e.g. males from Mourvèdre) across different mating ranks and females were less motivated to mate with the 'lower quality' males from Mourvèdre. Moreover, females mated with virgin males (i.e. their first mating) had a greater fecundity than females mated with nonvirgin males (i.e. their subsequent matings), regardless of the male's larval origin. Thus, females were more motivated to mate with virgin males which had high spermatophore quality than with nonvirgin males, which transferred less nutritive substances and fewer sperm at mating. Our results suggest that 'male quality' depended on both male larval origin and mating history, and had major consequences for female reproductive output. Moreover, females were able to discriminate between these males, and to receive large direct benefits they were more motivated to mate with males that had high sperm quantity (virgin males or males from certain cultivars or geographical locations).

### *Nutrition, Male Mating Capacity and Lifetime Reproductive Investment*

For each mating opportunity, the mating success (i.e. the probability of a male acquiring a mate) of males did not vary according to their larval origin, and remained high and constant over successive matings (minimum 80%; maximum 100%), suggesting that almost all males had an unlimited mating capacity until their death (based on an intermating recovery period of 24 h). However, their larval origin and pupal mass strongly influenced male longevity, with small males reared on Carignan in Perpignan living for a shorter period than large males from Grenache; consequently, these males had fewer mating opportunities over their lifetime in natura. This may be partly related to the pupal mass because smaller males have less energy reserves than larger males (Muller, Teixeira-Brandao, et al., 2016), and cannot afford to invest in both somatic maintenance and reproductive effort (Boggs, 2009; Boggs & Freeman, 2005). Indeed, males in good condition may be better competitors and have generally better mating success than males in poor condition, without incurring any survival cost (Engqvist, 2011; Grandison, Piper, & Partridge, 2009). Indeed, these males had more energy reserves to invest in somatic maintenance and/or reproduction and are expected to outcompete smaller males reared on less nutritive host plants. We found that the number of male matings was positively related to male longevity: the longer a male lived, the better its chance of reproducing several times (Molleman, Ding, Boggs, Carey, & Arlet, 2009). Our results suggest that males from Grenache in Estézargues and Sénas were in the best condition, investing in both somatic maintenance (lived for >8 days) and reproduction (had the largest number of copulations).

### *Male Quality: Effect of Nutrition and Mating History*

Male reproductive investment, besides depending on the male's larval nutrition, markedly decreased with increasing number of copulations, which was largely because of the male's inability to replenish resources in the adult stage. Between the first and subsequent matings of males, there was a more than 60% decrease in spermatophore volume, confirming that male spermatophore production is very costly (Vahed, 1998). Thus, in *L. botrana* and more generally in capital breeder species, males have only a single nutrient-rich spermatophore, which is produced using energy reserves derived from larval nutrition. We previously demonstrated in a laboratory strain of *L. botrana* that the first spermatophore of males plays a crucial role in egg production (Muller, Arenas, et al., 2016; Muller, Teixeira-Brandao, et al., 2016), and the present study confirms this finding in wild populations of *L. botrana*. This is consistent with the general assumption that male multiple mating can result in the depletion of specific ejaculate components, resulting in decreased fecundity and fertility of their mates (Perez-Staples, Aluja, Macías-Ordóñez, & Sivinski, 2008; Wigby et al. 2009). However, our results also indicate that the second spermatophore delivered by *L. botrana* males was 66–80% smaller than the first (according to their larval origin) but the fecundity of the female mated to a once-mated male decreased by only 5–40% according to male larval origin. This suggests that spermatophore size may not be a reliable predictor of female fecundity, and that the quality of the spermatophore rather than its quantity might better explain the variation observed in female fecundity (Bissoondath, & Wiklund, 1996; Muller et al. 2015). Both the number of sperm and/or the composition of the ejaculate (e.g. accessory gland secretions) can affect female fecundity (reviewed by Perry, Sirot, & Wigby, 2013), and we recently found that protein-derived spermatophores are a key factor in female reproductive output (Muller, Teixeira-Brandao, et al., 2016).

The numbers of apyrene and eupyrene spermatozoids also decreased with increasing number of matings. Eupyrene and apyrene spermatogenesis is known to occur at different stages during moth development (Friedländer, Seth, & Reynolds, 2005). Eupyrene spermatogenesis typically begins during the later larval instar stages and ceases at pupation, while apyrene spermatogenesis usually starts just prior to pupation and continues throughout adulthood. All the *L. botrana* males sampled in this study emerged with a finite number of eupyrene sperm, and the males did not release all sperm during the first mating, but retained some for future mating opportunities, ensuring fertilization of the same proportion of female eggs over consecutive matings. However, female fertility was affected by male larval origin, with females mated with males from Mourvèdre having reduced fertility compared with females mated with males from Grenache or Carignan. Males from Mourvèdre were likely to have low-quality sperm, and although they transferred the same number of eupyrene sperm as males from the other cultivars, they were not able to fertilize more than 70% of female oocytes, regardless of their mating history. As with the spermatophore volume, the quantity of sperm (which is always in excess of the number of eggs) is probably a minor factor relative to its quality (Snook, 2005; Werner & Simmons, 2008). Numerous sperm traits that contribute to paternity (including sperm size, viability, and mobility) are known to influence fertilization efficiency in moths (Morrow & Gage, 2000; Perry et al. 2013). In *L. botrana*, male larval food composition could directly influence sperm quality, as demonstrated in other moth species (Cordes et al. 2015; Gage & Cook, 1994).

### *Implications for the Evolution of Female Mate Choice*

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

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

Second, the latency period to mating increased significantly with increasing number of matings, with nonvirgin males that had already mated taking 20–25% more time to mate than virgin males. This suggests that after their first mating, males needed more time to successfully mate. This may be because of cumulative fatigue resulting from successive mating, or because the females were able to detect that these males were potentially sperm-depleted, and were more reluctant to mate with them. Analysis of the precopulatory behaviours of each sex suggested that the amount of time required to mate by experienced males was not merely a result of cumulative fatigue, because males were equally active during courtship regardless of their mating history. Thus, the longer latency period prior to mating for nonvirgin males was probably the result of female reluctance to mate with previously mated males. Indeed, females were more motivated to mate with virgin males than with nonvirgin males. In a recent study of *L. botrana* involving mate choice experiments, we demonstrated a female preference for virgin males, which maximized the direct benefits associated with receiving large spermatophores (Muller, Arenas, et al., 2016; Muller, Teixeira-Brandao, et al., 2016).

Nevertheless, this study was a laboratory experiment and there is not yet any demonstrated evidence of the existence of female mate choice in natura in this species. Models usually predict that the level of female choosiness should depend on the importance of the cost of searching mates (i.e. the proportion of lifetime devoted to searching for mates) which depends on the operational sex ratio and the encounter rate (Bleu, Bessa-Gomes, & Laloi, 2011; Etienne, Rousset, Godelle, & Courtiol, 2014). Highly choosy females run the risk of remaining unmated and the level of choosiness is likely to reach a value that counterbalances the benefits of obtaining high-quality males and the costs of mating and of remaining unmated (Kokko & Mappes, 2005). Typically, females should mate fairly indiscriminately when they first mate because of the large fitness cost of not mating (Worthington & Kelly, 2016). However, in *L. botrana*, the occurrence of mating failures seems to be low (Torres-Vila, Rodriguez-Molina, McMinn, & Rodriguez-Molina, 2004) and our previous mate choice study (Muller, Arenas, et al., 2016; Muller, Teixeira-Brandao, et al., 2016) indicates that virgin females have evolved the capacity to discriminate between males based on male mating experience.

### *Conclusion and Future Perspectives*

Our results highlight the overall importance of larval nutrition in male mating capacity and lifetime reproductive investment, all of which could modulate the reproductive strategies of this pest. In *L. botrana* species, both sexes are expected to be choosy about their mating partners because both males and females vary greatly in their reproductive quality according to intrinsic and extrinsic conditions (Moreau et al. 2006; Muller et al. 2015). Indeed, males and females have nontrivial reproductive costs (Harari et al. 2011). First, females that invest many limited resources in egg production would be expected to preferentially mate with high-quality males (virgin males or males in good condition as a consequence of their larval nutrition) to obtain large direct benefits from mating (such as large and nutrient-rich spermatophores). Second, males are also expected to exhibit some mate choice, because spermatophore production is costly and males only produce one nutrient-rich spermatophore throughout their lifetime (Bonduriansky, 2001). Moreover, in this moth species, both sexes invest in mate-finding traits; *L. botrana* females emit costly pheromones to attract mates (Harari et al. 2011; Umbers, Symonds, & Kokko, 2015) and males actively search for mates by following these chemical signals. The occurrence of mate choice by both sexes of this species should be investigated further.

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