Male larval nutrition affects adult reproductive success in wild European grapevine moth (Lobesia botrana)

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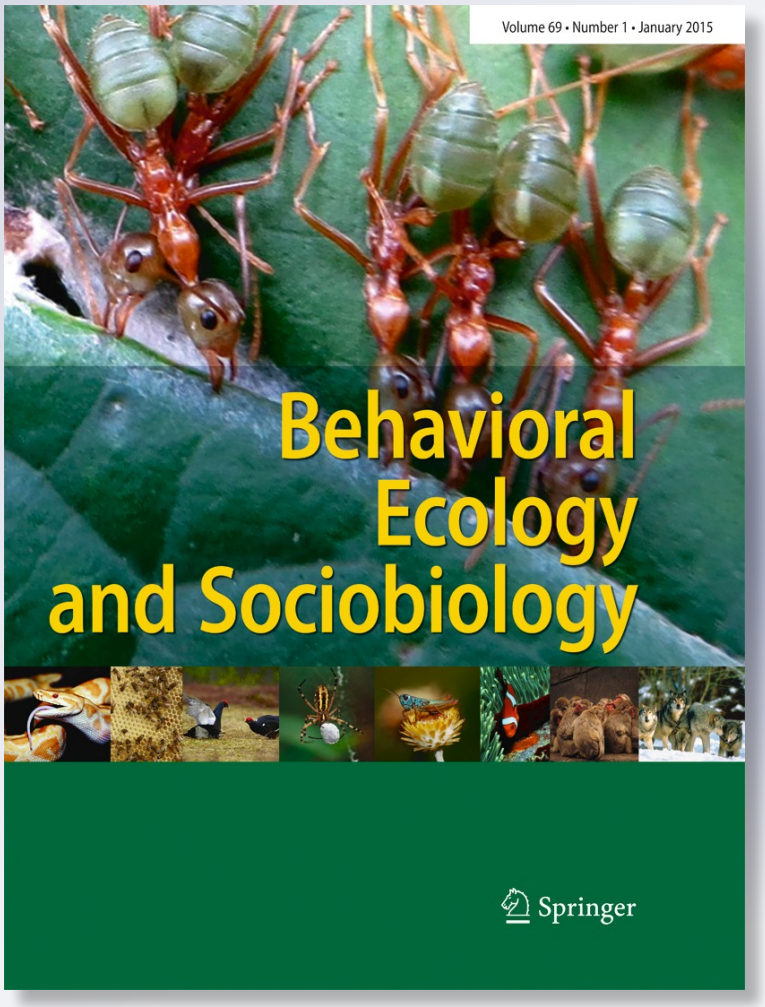

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ORIGINAL PAPER

Male larval nutrition affects adult reproductive success in wild European grapevine moth (*Lobesia botrana*)

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Abstract In Lepidoptera, males transfer a spermatophore to females containing sperm and accessory gland secretions that are reinvested into female reproduction, providing a fitness gain to females. One of the key factors shaping male spermatophore size is certainly the resources that males have acquired as larvae. In this study, we investigate how male larval food contributes to shaping the spermatophore quantity and quality and how it affects female reproduction in the European grapevine moth (*Lobesia botrana*). Specifically, we examined the effect of male origin (cultivar or geographical site) on their mating success by scoring individual motivation to mate, male spermatophore size and amount of sperm, and finally female fecundity and fertility. A strong effect of larval cultivar was found on spermatophore size and amount of fertilizing sperm produced by males. These male characteristics had important repercussions on female reproductive output. Females mating with males producing the biggest spermatophore and more fertilizing sperm were the most fecund and fertile. Finally, females were able to recognize males of different quality during the precopulatory phase and changed their mating behavior accordingly. The present results suggest that male nutritional quality could have an important implication for population dynamics.

Keywords Male and female reproductive output · Host plant · *Lobesia botrana* · Larval nutrition · Precopulatory behaviors · Spermatophore

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Introduction

In phytophagous insects, the variance in female reproductive output is often attributed in large part to the host plant on which these females have completed their larval development (for a review, see Awmack and Leather 2002). Typically, within one species, females that grow as larvae on "high-quality" plants (i.e., containing high levels of nutriments such as protein, carbohydrates, or lipids) are heavier and have a higher fecundity and longevity than females from "poor-quality" plants (Carrière 1992; Mevi-Schütz and Erhardt 2005; Moreau et al. 2006a). However, their reproductive output does depend on nutrients acquired not only from juvenile feeding but also from nuptial gifts provided by males during mating (Boggs 1990; Vahed 1998) which represent a significant male investment in the female and/or in her offspring (reviewed by Boggs 1995; Gwynne 2008). In Lepidoptera, males classically transfer a spermatophore to the female (Mann 1984) containing sperm and accessory gland secretions that could be reinvested into female reproduction. Indeed, in several butterflies, spermatophore-derived radiolabeled substances (such as amino acids, zinc, phosphorus, and sodium) were recovered into the female somatic tissue and eggs (reviewed by Vahed 1998). Moreover, it has been shown in several species that males transmit chemicals such as pyrrolizidine alkaloids or cyanogenic glycosides to the female with the spermatophore that help protect the female, her eggs, or both against predation (Eisner and Meinwald 1995; González et al. 1999; Cardoso

and Gilbert 2007). The spermatophore also contains fertile eupyrene sperm and non-fertile apyrene sperm. The eupyrene sperm comprises a nuclear material and serves to fertilize eggs, while the function of anucleate apyrene sperm remains unclear; also hypotheses suggest a role in sperm transport, nutrient donation, or sperm competition (Silberglied et al. 1984). There was no empirical evidence for the first two hypotheses in contrast to the latter which has been supported in *Pieris napi* (Pieridae) (Cook and Wedell 1999).

A recent meta-analysis showed that larger spermatophores enhance female fecundity but not their longevity in different insect orders, the Coleoptera, Lepidoptera, and Orthoptera (South and Lewis 2011). This analysis strongly suggests that spermatophore size is a key determinant of female reproductive output. It has long been recognized that the spermatophore is a costly physiological product (Oberhauser 1988; Cahenzli and Erhardt 2012). Thus, the size of the spermatophore depends on various factors: mating history of males (Bissoondath and Wiklund 1996; Torres-Vila and Jennions 2005), time elapsed since the last copulation (Lamunyon and Eisner 1994), male mass, age at the first mating (Oberhauser 1989; Tigreros 2013), and male larval or adult nutrition (Lederhouse et al. 1990; Delisle and Bouchard 1995). Indeed, nutrients acquired during both larval and adult stages can pass directly into the spermatophore, affecting its mass, size, and content as shown in *Papilio glaucus* (Lepidoptera: Papilionidae) in which a supplementation of adult diet with electrolytes and amino acids enhances male reproductive success (Lederhouse et al. 1990). The effect of adult nutrition on male spermatophores is now well documented (Lewis and Wedell 2007; Cahenzli and Erhardt 2012), but very few studies have examined the effect of larval nutrition on male spermatophore size (see Delisle and Bouchard 1995; Delisle and Hardy 1997 for two exceptions).

In most phytophagous Lepidoptera, males are pure capital breeders, i.e., their reproductive potential is limited by the nutrition ingested during their larval development. Larval nutrition is thus a critical factor explaining male adult reproductive success. For instance, a reduction in larval food quantity results in reduced adult body size with negative effects on male spermatophore size (Carroll 1994; Delisle and Hardy 1997). Moreover, in the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), males suffer resource restrictions as larvae reduce apyrene and eupyrene sperm numbers (Gage and Cook 1994), showing that the number of sperm could also be affected by male nutrition. Surprisingly, very little attention has been paid to the impact of variation in host plant quality on the mating success of males and on the consequences on female reproductive output. A fair hypothesis is that male reproductive performances (spermatophore size and sperm number) depend on the host plant on which they fed on as larvae because different host plants may have very different nutritional values. Assuming that spermatophore size and sperm number are strongly linked with the reproductive output of females, then fecundity and fertility of females should also vary across host plants on which their male partner grew. In that context, it is important to assess how (i) larval nutrition can affect male reproductive performances (spermatophore size and sperm number) and (ii) how the resulting performances of these males impact female reproductive output (fecundity, fertility, and longevity).

Assuming that males have different reproductive performances related to their larval nutrition on host plants and that female reproduction is modulated by male reproductive efficiency, we predict that mate choice based on phenotypic traits of male quality should have evolved in females. Male quality can be evaluated by female through his courtship display before mating. Hence, better males with bigger spermatophores can be expected to exhibit a stronger courtship display (like activity, latency to the first contact, Phelan and Baker 1990; Whittier et al. 1994) and thus would be accepted sooner by females. A way to measure female choice regarding male origin is to evaluate their mating motivation. In some species of moths and butterflies, females assume a typical calling position during which they emit pheromones. This calling performance could be used to quantify the motivation of females to copulate since the production of sex pheromone is costly (Harari et al. 2011). Therefore, we asked how precopulatory behaviors (reflecting male and female motivation to mate) could be adjusted according to partner quality mediated by host plant.

To summarize, our study aim was to assess in one natural population of moth the influence of larval diet on male reproductive performances and its consequences on female reproductive output. Moreover, male and female precopulatory behaviors were taken into account. The European grapevine moth (*Lobesia botrana*) (Lepidoptera: Tortricidae, Denis and Schiffermüller), a very important pest of grapes worldwide, is an ideal model to test this since this insect is a capital breeder and several studies have shown strong effects on female fitness in both lab strains (Savopoulou-Soultani et al. 1999; Thiéry and Moreau 2005; Moreau et al. 2006b, c) and in wild insects (Moreau et al. 2007). In addition, previous studies have evidenced that a variation in the phenology of one grape variety can affect male spermatophore volume, which may then impact female fecundity (Torres-Vila et al. 1999). However, no study to date has shown a cultivar effect on wild male *L. botrana*, which is the purpose of our study.

Materials and methods

Field sampling

Larvae of *L. botrana* were collected on June 2013 (corresponding to the first larvae generation of the year) in the field.

To test for a cultivar effect within a given population, larvae were sampled from three grape varieties (*Vitis vinifera* cv "Carignan," "Mourvèdre," and "Grenache") in the same vineyard ("Polygone" plot, Perpignan, France) to avoid potential bias due to an environmental variation between vineyards (temperature, light exposure, humidity, parasitism rate). To test for a geographical effect, larvae were sampled from three geographically different vineyards and thus populations growing on the same cultivar. To achieve this, larvae were, in addition, collected on the same cultivar Grenache from Estézargues (at 230 km east from Perpignan, France) and Sénas (at 290 km east from Perpignan and 60 km southeast from Estézargues, France). We sampled larvae of *L. botrana* at the end of their larval cycle (fifth instar) when building a glomerulae made of silk and flower buds (phenology 17–25, Eichhorn and Lorenz 1977). Classically in this pest species, most larvae accomplish their whole development in a single bunch. Larvae completed their life cycle in the laboratory in small polyethylene boxes (60×40 cm, height 21 cm) and fed ad libitum on bunches of the same cultivar sampled in the same place, at 22±1 °C, 60±10 % RH at a natural photoperiod. Larvae were checked daily until pupation, upon which pupae were gently extracted from glomerulae. Pupae were weighed to the nearest 0.01 mg on a Precisa 262 SMA-FR microbalance and placed individually in glass tubes (70×9-mm diameter) stoppered with cotton plugs, and then stored at 22 °C under a natural photoperiod. Pupae were checked every morning, and newly emerged adults sexed. Males were used for the following experiments and were randomly divided into two subsamples. The first subsample was used to measure male reproductive performances and the different adult precopulatory behaviors, and the second one was used to evaluate the consequences of male origin (cultivar and site) on the reproductive output of females. To only assess the effect of males on the reproductive performances of both sexes, the wild males were exposed to standardized females (see below) which were considered equivalent and thus randomly assigned to males.

Origin of laboratory female

In order to obtain standardized females, all females used in this study came from an inbred strain of *L. botrana* (INRA Bordeaux). The use of an inbred strain helps to minimize genetic variation between females and allows us to detect the male effect. The stock colony was maintained without diapause on a semi-artificial diet [1000 mL water, 15 g agar, 84.63 g maize flour, 41.25 g wheat germ, 45.48 g yeast, 6.03 g ascorbic acid, 3.35 g Wesson salts, 0.32 mL Scala, 5 mL ethanol (95 %), 2.65 g benzoic acid, 2.76 g Nipagin, as described by Thiéry and Moreau 2005], and maintained at 22±1 °C, 60±10 % relative humidity, and under a 16L:8D photoperiod. Males and females were placed in a large cage, and bands of waxed paper (15×2 cm) were hung for oviposition support. Once the paper received sufficient number of eggs, it was placed in a plastic box containing the larval semi-artificial diet. Larvae were checked daily until pupation and then were weighed to the nearest 0.01 mg on a Precisa 262 SMA-FR microbalance and placed individually in glass tubes (70×9-mm diameter) stoppered with cotton plugs. Larvae were checked every morning and newly emerged females were used for the experiment.

Precopulatory behaviors and reproductive performances of field collected males from different origins

At dusk, one 2-day-old virgin male from each origin (cultivar or site) was placed into a mating tube (100×15-mm diameter) with one 1–2-day-old virgin standardized female originating from the stock population. Male and female sexual activities were videotaped until mating (SONY HDR CX220E). Mating was considered as successful if the pair formation lasted more than 1 minute, which is the threshold over which genital coupling is completed. The onset time (time elapsed from the session's start until genital coupling) and the duration (time during which we observed a pair formation) of each mating were noted, and four behaviors that reflect female and male sexual motivations were recorded. We defined a parameter called "female motivation" that reflects the penchant of a female to mate. In this species, when a female is ready to mate (i.e., motivated to mate), she signals her readiness by releasing her sex pheromone. To do this, the female assumes a calling position with wings raised and pheromonal gland exposed. A female not intending to mate does not exhibit this typical calling behavior. So, (i) the onset time of calling (time elapsed from the session's start until females initiate calling) and (ii) the percentage of calling by females corresponding of the percentage of time in calling position (expressed by the time spent calling divided by the onset time of mating × 100) were noted. To evaluate mating ability and sexual vigor in courtship of males, we recorded (iii) the percentage of male activity (expressed by the time spent in movement by the male divided by the onset time of mating × 100) and (iv) the number of male mating attempts (corresponding to curved their abdomen and touched female abdomen without successful copulation).

Immediately after the end of mating, females were frozen at −25 °C for 10 min and then were dissected on a glass side. The bursa copulatrix containing the male spermatophore was removed in order to estimate his size. The spermatophore produced by male *L. botrana* is very small (less than 1 mg) and consequently difficult to accurately weigh. Estimating spermatophore size by extrapolating its volume is a classical method used in moths (Royer and McNeil 1993; Foster and Ayers 1996; Milonas et al. 2011) and in previous works on *L. botrana* (Torres-Vila et al. 1999) and we used it. To asses this size, dimensions (length *l*, width *w*, and thickness *t*) were

measured under a stereomicroscope (NIKON SMZ1500) with a magnification of ×20. The volume of the spermatophore was estimated as an ellipsoid balloon as in Torres-Vila et al. (1999) ($V=\pi/6\ (l\times w\times t)$) after preliminary measures to check that this process is repeatable ($n=47$; repeatability coefficient=0.863, Lessells and Boag 1987). Then, the sperm-containing ampulla was ruptured in a drop of distillated water, and the sperm mass was gently stirred to ensure dispersion. At this stage, eupyrene sperms were encysted in bundles and each bundle contains 256 eupyrene sperms in Lepidoptera (Virkki 1969; Phillips 1970; Cook and Gage 1995). Intact bundles were counted under a Nikon Eclipse E600 microscope at ×40/0.65 magnification, and the number of bundles was multiplied by 256 to give the total number of eupyrene sperms. The solution was then washed from the slide into a 1.5-mL centrifuge tube and diluted with distilled water. Four 10-μL subsamples were retrieved from each dilute sperm solution, and apyrene sperms were counted by scanning each dried drop under a Nikon Eclipse E600 microscope at ×100 magnification. The total number of apyrene sperm was estimated by multiplying the average sperm count (per four subsamples, coefficient of variation=14 %) by its dilution factor.

Male origin repercussions on reproductive output of standardized females

One 2-day-old virgin male originating from each cultivar and site was placed into a mating tube (100×15-mm diameter) with one 1–2-day-old virgin standardized female originating from the stock population at dusk. After one successful mating (separation of the pair), males were removed and females were held in the mating tube with water ad libitum. These females were allowed to oviposit freely on the inside surface of the glass tube until their death. Female survival was checked daily, and after the female's death, the eggs were incubated under the same conditions as moth maintenance during 7 days. We recorded (i) achieved fecundity (mean number of eggs laid per female), (ii) female fertility (proportion of hatched eggs), and (iii) longevity.

Statistical analysis

All statistical tests were performed using R software version 3.0.0 (R Core Team 2013). For each analysis, we report the full model deleting insignificant interactions, following Forstmeier and Schielzeth (2011). To assess the mating success of males (percentage of successful mating) according to their larval origin (cultivar and site), we used Pearson's χ^2 test. The effect of male origin (cultivar and site) on male pupal mass was tested with one-way ANOVA followed by Tukey's test. The effect of male origin (cultivar and site) on precopulatory behaviors of males (percentage of activity) and females (onset time of calling and percentage of calling), on the onset time of mating and the time spent mating, on the spermatophore volume transferred by males, and on the female fecundity was analyzed with ANCOVAs (respectively, with the pupal mass of males and females as covariates). Because data on sperm and male mating attempts were counted and were over-dispersed, a generalized linear model with a negative binomial distribution (NBGLM) was used to assess the effect of male origin on sperm and mating attempts. A GLM using a quasi-binomial error structure and a logit link function was used to analyze the proportion of hatched eggs by females mated with males from different origins (Warton and Hui 2011). Finally, Cox regression was applied to assess the influence of male origin, female pupal mass, and fecundity on female longevity.

Results

Male pupal mass

Male pupal mass varied significantly according to the origin (cultivar and site) of the field larvae ($F_{4,217}=24.22$, $p<0.0001$). In a given site (Perpignan), males from Mourvèdre were larger than those from Carignan (Table 1). Between different sites, males from Grenache in Estézargues and Sénas were heavier than those from Grenache in Perpignan (Table 1).

Mating success, mating duration, and precopulatory behaviors

The mating success of virgin *L. botrana* males (proportion of pairs for which genital coupling was observed more than 1 minute) did not vary according to their origin ($\chi^2_4=4.90$, $p=0.30$), between 72 and 88 % of males having successfully achieved their mating (Table 1). The onset time of calling was affected neither by male origin (cultivar or site) nor by female or male pupal mass (Table 1 and 2). However, the percentage of calling (expressed by the time spent calling divided by the onset time of mating × 100), which is an indication of female motivation, was affected by the male cultivar (Table 1 and 2). Females in the presence of males from Mourvèdre spent about 20 % of their time in calling position and therefore were less motivated than females with males of other cultivars which spent about 40 % of their time in calling position (Table 1). The percentage of activity by males was not affected by the cultivar on which they fed as larvae but was negatively related to their pupal mass (Table 2). There was no effect of male origin or male pupal mass on the number of attempts a male performed before successfully mating with a female (Table 1 and 2).

The time spent mating varied among larval cultivars and was negatively affected by male pupal mass (Table 2).

Table 1 Mating success and pupal mass of males of *L. botrana* depending on their origin (cultivars and sites) as well as male and female precopulatory behaviors and mating duration for each moth pair.

			Mating duration		Male behaviors		Female behaviors	
Cultivar	Mating success[a] (%)	Male pupal mass[b] (mg)	Onset time of mating[c] (min)	Time spent mating[c] (min)	Percentage of activity[c] (%)	Number of attempts[d]	Onset time of calling[c] (s)	Percentage of calling[c] (%)
Carignan (P)	85.19	5.06±0.11 (a)	11.2±2.0	61.3±3.9 (ab)	51.6±6.1	9.2±1.9	180.1±34.2	40.7±6.0 (a)
Mourvèdre (P)	88.16	5.52±0.06 (b)	9.3±2.3	50.3±1.7 (b)	39.9±4.5	4.7±0.6	158.8±18.9	21.1±3.9 (b)
Grenache (P)	72.41	5.19±0.10 (ab)	14.5±5.0	66.5±4.5 (a)	51.2±6.6	8.4±2.7	212.9±57.5	36.8±8.6 (ab)
Grenache (S)	88.09	6.27±0.06 (c)	12.9±3.6	63.5±4.0 (a)	45.7±5.8	6.1±0.8	167.3±39.6	43.0±4.9 (a)
Grenache (E)	81.58	5.91±0.09 (c)	10.5±2.7	68.5±4.5 (a)	41.8±7.9	6.0±1.1	114.1±41.9	45.1±5.2 (a)

The capital letters in brackets correspond to the different sites: (P) Perpignan, (S) Sénas, and (E): Estézargues. Values in each column with the same lowercase letter are not significantly different ($p>0.05$)

[a] Pearson χ^2

[b] ANOVA

[c] ANCOVA

[d] NBGLM

Copulation duration of males from Mourvèdre was shorter than those of males from Grenache (Table 1). However, the onset time of mating was not affected by larval origin or by male or female pupal mass (Table 1 and 2).

Male spermatophore volume and consequences on female fecundity and longevity

The volume of the spermatophore transferred by males during their first mating was correlated with their pupal mass and depended on their larval origin (ANCOVA, larval origin effect: $F_{4,87}=6.85$, $p<0.0001$; male mass effect: $F_{1,87}=13.83$, $p=0.0003$; female mass effect: $F_{1,87}=1.87$, $p=0.17$; Fig. 1a). Males emerged from Mourvèdre and Carignan produced a smaller spermatophore compared to males from Grenache. There was no geographical effect on the volume of the spermatophore transferred by males from the same cultivar on three different sites (Fig. 1a). The number of eggs laid by females depends on the male with which they mate and is also positively related to female pupal mass (ANCOVA, larval origin effect: $F_{4,99}=5.44$, $p=0.0008$; female mass effect: $F_{1,99}=11.48$, $p=0.001$; Fig. 1b). Females mated with males from Mourvèdre (the smallest spermatophores) laid fewer eggs than females mated with males from Grenache (males with the biggest spermatophores; Fig. 1a, b). Surprisingly, males raised on Carignan produced a small spermatophore which still allows the females to lay as much eggs as the females that received a bigger spermatophore (mated with males from Grenache). Moreover, there was no geographic effect on the fecundity of females mated with males from Grenache originating from the different sites (Fig. 1b). By contrast, female longevity, ranged from 10.1 to 11.1 days, was not affected by the origin of the male with which they mated but was positively related to female pupal mass (Cox

Table 2 Cultivar and male and female pupal mass effects on female behaviors (onset time of calling and percentage of calling), male behaviors (percentage of activity and number of mating attempts), onset time of mating, and mating duration

	Mating duration				Male behaviors				Female behaviors			
	Onset time of mating[a]		Time spent mating[a]		Percentage of activity[a]		Number of attempts[b]		Onset time of calling[a]		Percentage of calling[a]	
Source	Test value	*p*	Test value	*p*	Test value	*p*	Test value	*p*	Test value	*p*	Test value	*p*
Cultivar	$F_{4,77}=0.35$	0.84	$F_{4,77}=5.33$	*<0.001*	$F_{4,66}=0.74$	0.57	LR=7.39	0.12	$F_{4,66}=1.34$	0.27	$F_{4,66}=4.33$	*0.01*
Male mass	$F_{1,77}=0.51$	0.82	$F_{1,77}=4.69$	0.003	$F_{1,66}=4.55$	*0.04*	LR=1.34	0.25	$F_{1,66}=2.91$	0.09	$F_{1,66}=0.67$	0.42
Female mass	$F_{1,77}=0.10$	0.76	$F_{1,77}=0.01$	0.90	$F_{1,66}=0.69$	0.41	LR=0.78	0.38	$F_{1,66}=0.72$	0.72	$F_{1,66}=0.61$	0.44

p values <0.05 are shown in italics

[a] ANCOVA

[b] NBGLM

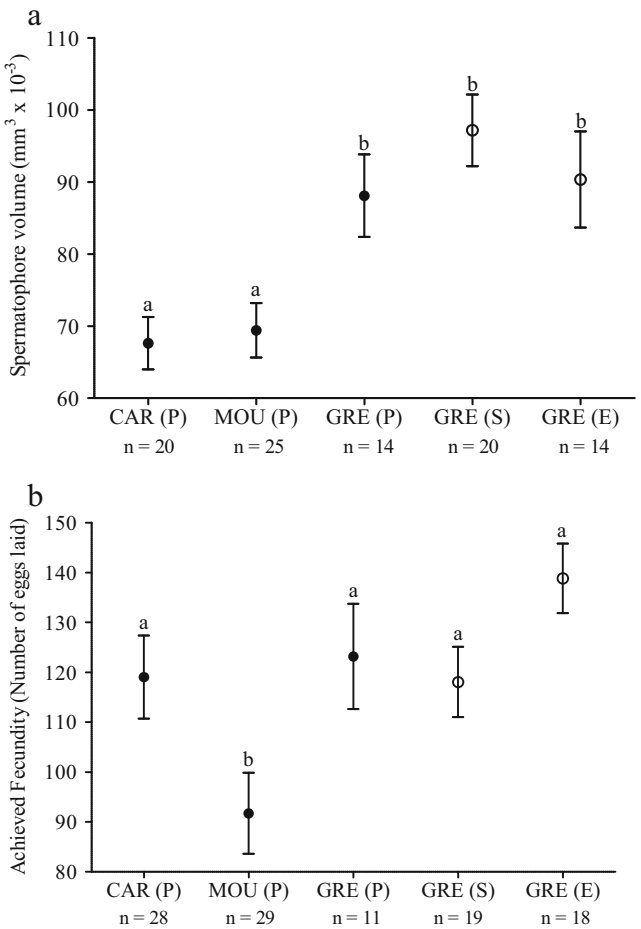

Fig. 1 **a** Spermatophore volume (mean±SEM) of *L. botrana* males fed as larvae on different cultivars and sites and **b** consequences on female achieved fecundity (mean±SEM) mated with males from these different origins: cultivars (*black circles*) and sites (*white circles*). The *capital letters* in brackets correspond to the different sites: (*P*) Perpignan, (*S*) Sénas, and (*E*) Estézargues. *Acronyms* correspond to the different cultivars: *CAR* cv "Carignan," *GRE* cv "Grenache," and *MOU* cv "Mourvèdre." *Numbers* at the bottom of each column represent the sample size

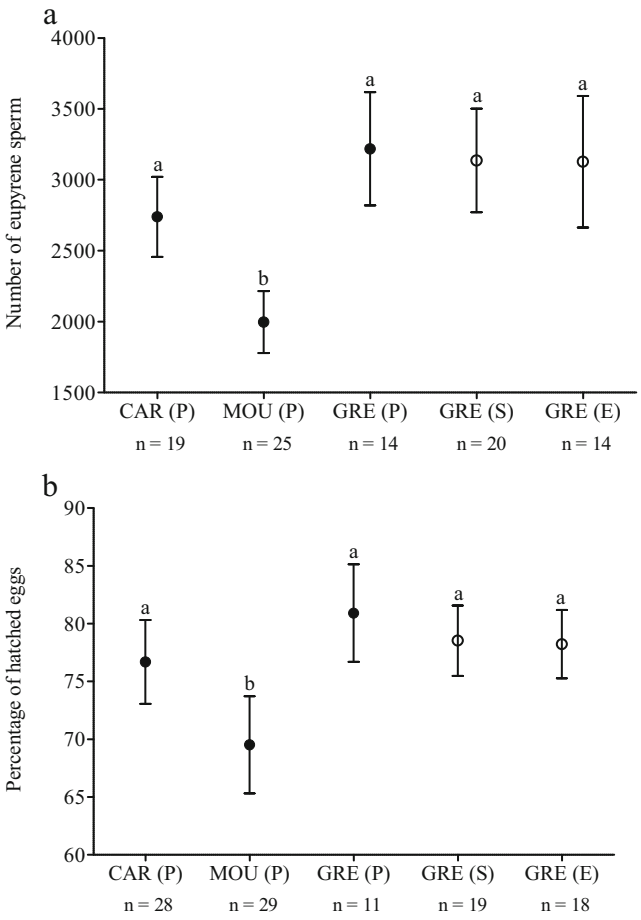

Fig. 2 **a** Eupyrene sperm number (mean±SEM) of *L. botrana* males fed as larvae on different cultivars and sites and **b** consequences on female fertility (percentage of hatched eggs, mean±SEM) mated with males from these different origins: cultivars (*black circles*) and sites (*white circles*). The *capital letters* in brackets correspond to the different sites: (*P*) Perpignan, (*S*) Sénas, and (*E*) Estézargues. *Acronyms* correspond to the different cultivars: *CAR* cv "Carignan," *GRE* cv "Grenache," and *MOU* cv "Mourvèdre." *Numbers* at the bottom of each column represent the sample size

regression, $\chi^2_{2,99}=14.12$, $p=0.015$) and negatively correlated to female fecundity (Cox regression, $\chi^2_{2,99}=11.77$, $p=0.038$).

Male eupyrene sperm and consequences on female fertility

The number of eupyrene sperm produced by males depended on the cultivar on which they grew up. In Perpignan, males from Mourvèdre produced and transferred less eupyrene sperm than males from Carignan or Grenache (NBGLM, larval origin effect: LR=10.46, $p=0.03$; male mass effect: LR=0.04, $p=0.83$; Fig. 2a). The proportion of eggs hatched depended on the male with which females mated (GLM with quasi-binomial distribution, $p=0.02$). Females mated with males from Mourvèdre, which produced fewer eupyrene sperm, had fewer hatched eggs than females mated with males from other cultivars (Fig. 2a, b).

However, the number of non-fertilizing apyrene sperm, ranged from 39,183 to 48,259 sperms, was not affected by the origin (cultivar or site) of males and was not related to their pupal mass (NBGLM, respectively, LR=0.91, $p=0.98$; LR 0.27, $p=0.60$).

Finally, the number of eupyrene sperm and apyrene sperm were both positively related to the volume of the spermatophore [Spearman, respectively, $\rho=0.38$, IC95% (0.20; 0.54), $p=0.0002$; $\rho=0.22$, IC95% (0.01; 0.42), $p=0.0427$].

Discussion

Our results provide experimental evidence that larval nutrition on different grape cultivars affects male reproductive

performances in the European grapevine moth. This effect appears to be relatively strong because it was not modified according to the three geographical origins (from Grenache), except for pupal mass. The pupal mass, the volume of the spermatophore, and the number of fertilizing sperm transferred by males were affected by the cultivar on which they grew up. These male performances, related with larval nutrition, had important repercussions on female reproductive output. Indeed, females mated with males producing the biggest spermatophore and more fertilizing sperm (e.g. males from Grenache) had a higher fecundity and fertility than females mated with "low spermatic quality" males (e.g. males from Mourvèdre). Moreover, the time spent mating and the female motivation for copulating (assessed by the percentage of female calling) also depended on the male's origin, suggesting that females could modulate their behaviors in response to different male spermatic qualities.

The mating success of virgin males was relatively high (above 80 % for all cultivars and locations), confirming that almost all *L. botrana* males were sexually mature 2 days after their adult emergence, whatever their origin is. Although most males mated with a female, they did not have similar reproductive performances (spermatophore size and sperm numbers) according to their larval cultivar. This may be partly related to their pupal mass because larger males have more energetic and metabolic reserves than smaller males and can produce bigger spermatophores (Svärd and Wiklund 1989; Royer and McNeil 1993; LaMunyon and Eisner 1994). The differences in spermatophore size between males had important repercussions on female fecundity, an effect reported in numerous studies (LaMunyon and Eisner 1994; Wedell and Karlsson 2003; McNamara et al. 2009). In the present experiments, females mated with males from Mourvèdre, which transferred the smallest spermatophore, laid fewer eggs than females mated with males from Carignan or Grenache. This is in agreement with a recent meta-analysis led on 38 Lepidoptera species which demonstrated that the larger the ejaculate is, the more eggs the female lays (South and Lewis 2011). However, males raised from Carignan produced a small spermatophore which still allows the females to lay as much eggs as the females that received a bigger spermatophore. This suggests that the volume of the spermatophore is not the only factor influencing female fecundity. Rather than quantity, the quality of the spermatophore might better explain the variation observed in female fecundity (Marshall and McNeil 1989; Bissoondath and Wiklund 1996). An analysis of male spermatophore for nutrients such as protein content of males raised from different cultivars would be interesting. Finally, female longevity was not directly related to male quality which indicates that spermatophore would not play a significant role in female longevity. This is consistent with the meta-analysis cited above (South and Lewis 2011). However, female longevity was positively correlated with her pupal mass and negatively with her fecundity, a link that has already been observed in *L. botrana* females (Moreau et al. 2006a). These results are consistent with the well-documented trade-off between fecundity and longevity reported in a several insect species (for a review, see Jervis et al. 2007).

Moreover, the results also showed that larval food influenced the number of eupyrene-fertilizing sperm transferred during mating but not the apyrene sperm. Apyrene and eupyrene sperm numbers were both positively related to the volume of the spermatophore. These two positive correlations are not universal and probably depend on the moth or butterfly species. Our results are in agreement with a study on the armyworm *Pseudatelia separata* (He and Miyata 1997), but no correlation between spermatophore and eupyrene sperm has been found in other works (Cook and Wedell 1996, 1999). The existence of an effect of larval food only on eupyrene sperm number could be explained by the fact that eupyrene sperms are produced during larval stages until male pupation and are not renewed in the adult stage while apyrene sperms are produced just before pupation and continue to be produced during adult life (Friedländer 1997; Friedländer et al. 2005). Globally, our results contrast with studies that have not found any link between larval nutrition and sperm production (Lewis et al. 2011; Velde et al. 2013). These differences in eupyrene sperm numbers related to male origin may have a major impact on female fertility. Indeed, females mated with males which produced a higher number of eupyrene sperm had a higher number of fertilized eggs (i.e., fertility) than females mated with males which had the lowest level of these fertilizing sperm. Additionally, it is possible that all males produce sufficient sperms to fecund all female eggs, but their spermatophore could contain inadequate levels of ions that are known to negatively affect sperm motility (Khan and Musgrave 1969; Leopold 1976).

Here, we clearly showed that males had very different performances according to their larval cultivar. Furthermore, male origin also had repercussions on female precopulatory behaviors. Interestingly, females mated with "low-quality" males (from Mourvèdre) spent less time in calling position before copulation than females mated with "high-quality" males (from Carignan and Grenache). This indicates that females can recognize their mate quality before or during the precopulatory phase. It has been recently demonstrated that the pheromone production is costly for *L. botrana* females (Harari et al. 2011), and females signaling by sex pheromone production bear a cost and thus calling may serve to reflect the female motivation to copulate if these females are able to discriminate among males of different qualities. In this study, male origin did not affect male sexual vigor to mate suggesting that female preferences are not based on male courtship displays but probably depends on the "chemical fingerprint" of males which can differ according to their origin. Furthermore, the copulation duration was shorter for females

mated with low-quality males (from Mourvèdre). Some studies have suggested that copulation duration was under male control (Barbosa 2011) and depended on male's ability to produce a spermatophore (Wedell and Cook 1999). In our study, the time spent mating did not appear to be the result of a small spermatophore production. To better understand why we observed these differences in mating duration, it would be interesting to know when the spermatophore is transferred into the female bursa copulatrix, at the beginning or the end of mating. Given the importance of male quality (spermatophore size and sperm numbers) on female reproductive success (fecundity and fertility), one would expect *L. botrana* females to exhibit some degree of mate choice. Indeed, some phenotypic indicators of male quality such as male pupal mass are known to impact male precopulatory success, larger males being more chosen by females and getting advantages through male-male competition (Hunt et al. 2009). Our study suggests that females might be more or less motivated to mate according to the origin of male and paves the way for a potential degree of female mate choice *in natura*, based on, for instance, the chemical fingerprints of individuals.

In summary, this study demonstrated a strong effect of larval nutrition on male reproductive performances of the European grapevine moth, *L. botrana*. These male performances have important repercussions on motivation to mate and reproductive output of females. Our results suggest that the European grapevine moth would be an excellent experimental system to examine mate choice by both sexes because the variation found in male and female reproductive performances is partly explained by their larval nutrition and may lead to male and female mate choice, mediated by their larval cultivars. We suggest that studies on reproductive output in phytophagous females should not focus solely on female-host plant relationships but also take into account the effect of male reproductive performances on female reproductive output.

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References

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Ann Rev Entomol 47:817–844

Barbosa F (2011) Copulation duration in the soldier fly: the roles of cryptic male choice and sperm competition risk. Behav Ecol 22: 1332–1336

Bissoondath CJ, Wiklund C (1996) Effect of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). Funct Ecol 10:457–464

Boggs CL (1990) A general model of the role of male-donated nutrients in female insects' reproduction. Am Nat 136:598–617

Boggs CL (1995) Male nuptial gifts: phenotypic consequences and evolutionary implications. In: Leather SR, Hardie J (eds) Insect reproduction. CRC Press Inc, Florida, pp 215–242

Cahenzli F, Erhardt A (2012) Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. Oikos 121:1417–1423

Cardoso MZ, Gilbert LE (2007) A male gift to its partner? Cyanogenic glycosides in the spermatophore of longwing butterflies (*Heliconius*). Naturwissenschaften 94:39–42

Carrière Y (1992) Host plant exploitation within a population of a generalist herbivore, *Choristoneura rosaceana*. Entomol Exp Appl 65:1–10

Carroll AL (1994) Interactions between body size and mating history influence the reproductive success of males of a tortricid moth, *Zeiraphera canadensis*. Can J Zool 72:2124–2132

Cook PA, Gage MJ (1995) Effects of risks of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). Behav Ecol Sociobiol 36:261–268

Cook PA, Wedell N (1996) Ejaculate dynamics in butterflies: a strategy for maximizing fertilization success? Proc R Soc Lond 263:1047–1051

Cook PA, Wedell N (1999) Non-fertile sperm delay female remating. Nature 397:486–486

Delisle J, Bouchard A (1995) Male larval nutrition in *Choristoneura rosaceana* (Lepidoptera: Tortricidae): an important factor in reproductive success. Oecologia 104:508–517

Delisle J, Hardy M (1997) Male larval nutrition influences the reproductive success of both sexes of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Funct Ecol 11:451–463

Eichhorn KW, Lorenz DH (1977) Phaenologische Entwicklungsstadien der Rebe. Nachr bl. Dtsch Pflanzenschutzd 29:119–120

Eisner T, Meinwald J (1995) The chemistry of sexual selection. Proc Natl Acad Sci U S A 92:50–55

Foster SP, Ayers RH (1996) Multiple mating and its effects in the lightbrown apple moth, *Epiphyas postvittana* (Walker). J Insect Physiol 42:657–667

Forstmeier W, Schielzeth H (2011) Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. Behav Ecol Sociobiol 65:47–55

Friedländer M (1997) Control of the eupyrene–apyrene sperm dimorphism in Lepidoptera. J Insect Physiol 43:1085–1092

Friedländer M, Seth RK, Reynolds SE (2005) Eupyrene and apyrene sperm: dichotomous spermatogenesis in Lepidoptera. Adv Insect Physiol 32:206–308

Gage MJG, Cook PA (1994) Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). Funct Ecol 8:594–599

González A, Rossini C, Eisner M, Eisner T (1999) Sexually transmitted chemical defense in a moth (*Utetheisa ornatrix*). Proc Natl Acad Sci U S A 96:5570–5574

Gwynne DT (2008) Sexual conflict over nuptial gifts in insects. Ann Rev Entomol 53:83–101

Harari AR, Zahavi T, Thiéry D (2011) Fitness cost of pheromone production in signaling female moths. Evol 65:1572–1582

He Y, Miyata T (1997) Variations in sperm number in relation to larval crowding and spermatophore size in the armyworm, *Pseudaletia separata*. Ecol Entomol 22:41–46

Hunt J, Breuker CJ, Sadowski JA, Moore AJ (2009) Male–male competition, female mate choice and their interaction: determining total sexual selection. J Evol Biol 22:13–26

Jervis MA, Boggs CL, Ferns PN (2007) Egg maturation strategy and survival trade-offs in holometabolous insects: a comparative approach. Biol J Linnean Soc 90:293–302

Khan NR, Musgrave AJ (1969) Observations on the functional anatomy of the reproductive organs of *Sitophilus* (Coleoptera: Curculionidae). Can J Zool 47:665–669

LaMunyon CW, Eisner T (1994) Spermatophore size as determinant of paternity in an arctiid moth (*Utetheisa ornatrix*). Proc Natl Acad Sci U S A 91:7081–7084

Lederhouse RC, Ayres MP, Scriber JM (1990) Adult nutrition affects male virility in *Papilio glaucus* L. Funct Ecol 4:743–751

Leopold RA (1976) The role of male accessory glands in insect reproduction. Ann Rev Entomol 21:199–221

Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. Auk 104:116–121

Lewis Z, Wedell N (2007) Effect of adult feeding on male mating behaviour in the butterfly, *Bicyclus anynana* (Lepidoptera: Nymphalidae). J Insect Behav 20:201–213

Lewis Z, Sasaki H, Miyatake T (2011) Sex starved: do resource-limited males ensure fertilization success at the expense of precopulatory mating success? Anim Behav 81:579–583

Mann T (1984) Spermatophores: development, structure, biochemical attributes and role in the transfer of spermatozoa. Springer, Berlin

Marshall LD, McNeil JN (1989) Spermatophore mass as an estimate of male nutrient investment: a closer look in *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). Funct Ecol 3:605–612

McNamara KB, Elgar MA, Jones TM (2009) Large spermatophores reduce female receptivity and increase male paternity success in the almond moth, *Cadra cautella*. Anim Behav 77:931–936

Mevi-Schütz J, Erhardt A (2005) Amino acids in nectar enhance butterfly fecundity: a long-awaited link. Am Nat 165:411–419

Milonas PG, Farrell SL, Andow DA (2011) Experienced males have higher mating success than virgin males despite fitness costs to females. Behav Ecol Sociobiol 65:1249–1256

Moreau J, Benrey B, Thiéry D (2006a) Grape variety affects larval performance and also female reproductive performance of the European grapevine moth (*Lobesia botrana*). Bull Entomol Res 96:205–212

Moreau J, Benrey B, Thiéry D (2006b) Assessing larval food quality for phytophagous insects: are facts as simple as it appears? Funct Ecol 20:592–600

Moreau J, Arruego X, Benrey B, Thiery D (2006c) Differences in nutritional quality of parts of *Vitis vinifera* berries affect fitness of the European grapevine moth. Entomol Exp Appl 119:93–99

Moreau J, Thiéry D, Troussard JP, Benrey B (2007) Grape variety affects female but also male reproductive success in wild European grapevine moths. Ecol Entomol 32:747–753

Oberhauser KS (1988) Male monarch butterfly spermatophore mass and mating strategies. Anim Behav 36:1384–1388

Oberhauser KS (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. Behav Ecol Sociobiol 25: 237–246

Phelan PL, Baker TC (1990) Comparative study of courtship in twelve phycitine moths (Lepidoptera: Pyralidae). J Insect Behav 3:303–326

Phillips DM (1970) Insect sperm: their structure and morphogenesis. J Cell Biol 44:243–277

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Royer L, McNeil JN (1993) Male investment in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae): impact on female longevity and reproductive performance. Funct Ecol 7:209–215

Savopoulou-Soultani M, Nikolaou N, Milonas PG (1999) Influence of maturity stage of the grape berries on the development of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae. J Econ Entomol 92:551–556

Silberglied RE, Shepherd JG, Dickinson JL (1984) Eunuchs: the role of apyrene sperm in Lepidoptera? Am Nat 123:255–265

South A, Lewis SM (2011) The influence of male ejaculate quantity on female fitness: a meta-analysis. Biol Rev 86:299–309

Svärd L, Wiklund C (1989) Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. Behav Ecol Sociobiol 24:395–402

Thiéry D, Moreau J (2005) Relative performance of European grapevine moth (*Lobesia botrana*) on grapes and other hosts. Oecologia 143: 548–557

Tigreros N (2013) Linking nutrition and sexual selection across life stages in a model butterfly system. Funct Ecol 27:145–154

Torres-Vila LM, Jennions MD (2005) Male mating history and female fecundity in the Lepidoptera: do male virgins make better partners? Behav Ecol Sociobiol 57:318–326

Torres-Vila LM, Rodriguez-Molina MC, Roehrich R, Stockel J (1999) Vine phenological stage during larval feeding affects male and female reproductive output of *Lobesia botrana* (Lepidoptera: Tortricidae). Bull Entomol Res 89:549–556

Vahed K (1998) The function of nuptial feeding in insects: a review of empirical studies. Biol Rev 73:43–78

Velde LV, Schtickzelle N, Van Dyck H (2013) Effect of larval food stress on male adult behaviour, morphology and reproductive investment in the butterfly *Pararge aegeria*. Evol Ecol 27:221–234

Virkki N (1969) Sperm bundles and phylogenesis. Z Zellforsch Mikrosk Anat 101:13–27

Warton DI, Hui FK (2011) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10

Wedell N, Cook PA (1999) Strategic sperm allocation in the small white butterfly *Pieris rapae* (Lepidoptera: Pieridae). Funct Ecol 13:85–93

Wedell N, Karlsson B (2003) Paternal investment directly affects female reproductive effort in an insect. Proc R Soc Lond 270:2065–2071

Whittier TS, Nam FY, Shelly TE, Kaneshiro KY (1994) Male courtship success and female discrimination in the Mediterranean fruit fly (Diptera: Tephritidae). J Insect Behav 7:159–170