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ORIGINAL ARTICLE

Sexual audience affects male's reproduction investment without consequences on reproductive outputs

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Abstract Males evolved plastic strategies to respond to male–male competition and exhibit adaptive traits and behaviors maximizing their access to the females and limiting sperm competition. Mating behaviors allow males to express quick responses to current sexual audience, that is, the number of nearby conspecifics prone to mate. In contrast, physiological responses are frequently delayed because they are constrained by the time and resources having to be mobilized to produce and export sperm and associated products. This is especially critical in species for which males produce spermatophores. Here we investigated in what extend moth males (the tortricid moth *Lobesia botrana*) producing spermatophores exhibit plastic behavioral and physiological responses to different sexual audiences before and during mating and the consequences for their reproductive output. We found that males adjusted their mating behaviors and spermatophore size to a potentially elevated risk of sperm competition perceived before mating. In addition, males responded to the closed presence of females during mating by reducing their mating duration. Surprisingly, the various behavioral and physiological responses we highlighted here were not fully reflected in their reproductive performance as we did not reveal any effect on fecundity and fertility of their mate. The selective pressure exerted on males experiencing male–male competition could thus be sufficient to trigger adjustment in male mating behaviors but constrains physiological responses according to the perception of competition.

Key words *Lobesia botrana*; male–male competition; mating behavior; sexual conflict; sexual selection; sperm competition

Introduction

Accessing females, producing and transferring sperm to fertilize the eggs are costly for males (Dewsbury, 1982; Nakatsuru & Kramer, 1982; Scharf *et al*., 2013). Males thus employ strategies to optimize their mating frequency and fertilization success (Parker, 1978; Bonduriansky, 2001; Wedell *et al*., 2002; Louâpre *et al*., 2015). In polygamous mating systems, one of the main constrains males face to increase their paternity is their ability to bypass the access of females by competitors (or rivals) (Emlen & Oring, 1977). The presence of rivals is indeed a key determinant of the sexual environment leading to sometimes strong male–male competition to access females, and intense sperm competition within the female reproductive tract (Simmons, 2002). Risk models predict an increase of male reproductive investment for accessing the females and fertilizing the eggs when competing with rivals

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(Parker *et al*., 1997; Engqvist & Reinhold, 2005; Parker & Ball, 2005). In this regard, males express plastic response to male–male competition by perceiving direct cues, that is, the presence of rival males (Bretman *et al*., 2009), and indirect cues, those arising as a consequence of females mating by other males (Friberg, 2006). Therefore, males generally express adaptive reproductive strategies associated with a higher investment during and/or after mating (Wedell *et al*., 2002; Bretman *et al*., 2011a). For instance, they ejaculate more sperm (Gage & Baker, 1991; Gage & Barnard, 1996; Wedell & Cook, 1999; Thomas & Simmons, 2007; Bretman *et al*., 2011a; Garbaczewska *et al*., 2013), transfer more viable sperm (Magris, 2021), and seminal fluid proteins (Wigby *et al*., 2009) to the females. Such plastic responses to male–male competition also involves modifications of the mating behaviors such as a more intense harassment of females (Sih & Krupa, 1995) and an extended mating duration under strong competition (Friberg, 2006; Bretman *et al*., 2009).

Plastic male mating strategies are expected to be adjusted to the level of male–male competition they perceive at the appropriate timing (Parker *et al*., 1997; Engqvist & Reinhold, 2005). In a stable or predictably fluctuant sexual environments in which the intensity of the male–male competition to come is easily presaged, many male species are known to anticipate future reproductive competition during development though physiological, neural, and genomic mechanisms (Kasumovic & Brooks, 2011; Bretman *et al*., 2016). When males cannot easily anticipate the intensity of the male–male competition, as is frequently the case, they are expected to evolve behavioral strategies making them highly responsive to the sexual environment occurring at the time of mating (Kasumovic *et al*., 2008; Punzalan *et al*., 2010; Bretman *et al*., 2016). Indeed, mating behaviors allow males to express quick responses to current male–male competition in comparison with the time and resources having to be mobilized to produce and export sperm and associated products (Dewsbury, 1982; Wedell *et al*., 2002; Bretman *et al*., 2010, 2011b). Implementation of a physiological response to male–male competition sometimes explains the delayed development of juvenile males exposed to a higher risk of sperm competition allowing higher investment in testis development (Allen *et al*., 2011), and time lags between the perception of competition by mature males and the expression of the male responses (Rouse & Bretman, 2016).

The time required to produce, mature, and transfer viable sperm to females is amplified in taxa where males produce spermatophores, such as in Lepidoptera and Orthoptera (Mann, 2012). Spermatophore contains sperm and accessory gland secretions that could be reinvested into female reproduction (Vahed, 1998). It can also play an important role in interindividual sperm competition by increasing the length of the refractory period since it was shown that a bigger spermatophore increases the female latency to remate (McNamara *et al*., 2009). Given its content, spermatophore is thus a key determinant of the female reproductive output, and therefore, of the male fitness. We can predict a strong positive relationship between the level of male–male competition in Lepidoptera and the size of the spermatophore transferred to the female, as it was shown for example in Orthoptera (Simmons *et al*., 1993; Gage & Barnard, 1996). We also expect that the adjustment of the spermatophore size is a delayed response of males to the male–male competition, as their production is time and energy consuming (Muller *et al*., 2016).

Here, we used the European grapevine moth *Lobesia botrana* as a model system to investigate plastic responses to the risk of male–male competition and to what extend behavioral and physiological responses vary according to the sexual audience perceived before and during mating. In this species, polyandry is a heritable trait ($h^2 = 0.40 \pm 0.12$) (Torres-Vila *et al*., 2002) and is strongly associated with physiological factors, such as larval food nutrition (Torres-Vila *et al*., 2004; Thiéry *et al*., 2014a, 2014b) and the size of the spermatophore received by females (Torres-Vila *et al*., 1997). Spermatophore is highly plastic in its content and its shape. It depends on various factors such as mating history, time since the last copulation, physiological state, and food quality (see Muller *et al*., 2015 for a description of these factors). To assess the effect of reproductive competition, we measured the volume of the spermatophore, the duration of mating and the latency before mating of partners faced with different sexual audiences. We tested whether male can plastically adjust these traits to the presence of one or three rival males added to the mating arena 24 h before mating or during mating. We also added one or three supplementary females to the arena when the focal males engaged in copulation, in order to distinguish the plastic response of these focal males in presence of males or females.

Material and methods

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. Dissected females

were frozen at −25°C for 10 min in a freezer prior to decapitation.

Field sampling and housing of animals

Larvae of *L. botrana* were collected on June 2015 (corresponding to the first larvae generation of the year) at the end of their larval cycle (fifth instar) on floral clusters (grape phenology 17–25) (Eichhorn & Lorenz, 1977) in one vineyard planted with a single cultivar (*Vitis vinifera* cv Grenache, Senas plot, Roquemartine, France). Classically in this pest species, most larvae accomplish their whole development on a single grape stock or even 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 natural photoperiod (L17 : D6 and 1 h of dusk). Larvae were checked daily until pupation, and pupae were gently removed from the grape clusters. Pupae were weighed to the nearest 0.01 mg (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 natural photoperiod. Pupae were checked every morning, and newly emerged adults sexed.

General design

We performed two experiments for testing the ability of males to perceive and respond to the level of male–male competition prior (experiment 1) or during (experiment 2) mating. In the first experiment, virgin males were kept either alone, or by batch of two or four during 24 h, before individually exposed to a virgin female. This experiment allowed to test for the ability of males to respond to the male's density before encountering a female. In this experiment, conspecifics used before mating were only composed of males (not females), in order to avoid unwanted mating before exposing the focal males to virgin females. In the second experiment, a virgin female was proposed to a virgin male without prior male–male competition. Once the copulation started, either one or three supplementary virgin males were added to the mating chamber. This experiment allowed to test for a plastic response of the male engaged in copulation depending on the sexual audience. In this experiment, we also exposed some of the males to either one or three virgin females to assess the specific response of the male depending on the sex of the audience. For all matings occurring during the two experiments, half of the mated females were used to evaluate the male reproductive performance (i.e., spermatophore volume transferred to the female). The other half of the females allowed to evaluate the consequences of the male donation on the reproductive output of females (i.e., laying latency, fecundity, fertility). For all the experiments, only males from the field sampling were used. Females came from a laboratory breeding to minimize variance due to a female effect on the male behaviors (see Muller *et al*., 2015 for a detailed procedure of the female rearing and selection procedure). For the two competition experiments, the sample sizes for every modality are given in the corresponding figures.

Competition experiments

Experiment 1: Male–male competition prior to mating. The experiment started at dusk. One 2-day-old virgin male was placed into a plastic box (15 × 10 × 8 cm) either (i) alone (no competition treatment), (ii) with one 2-day-old virgin males (moderate competition treatment), or (iii) three 2-day-old virgin males (high competition treatment) during 24 h. At dusk of the next day, each male of each treatment was placed into a new plastic box (mating chamber hereafter) with one 2-day-old virgin female originating from the stock population. The male and female sexual activities were then observed continuously during the following 4 h. Mating was considered successful if the pair formation lasted more than 1 min, which is the threshold over which genital coupling is completed. Once mating finished, all the females engaged in pair were collected.

Experiment 2: Male–male competition during mating. The experiment started at dusk. One 2-day-old virgin male was placed into a mating plastic chamber (15 × 10 × 8 cm) with one 2-day-old virgin standardized female originating from the stock population for 4 h. During this session, male and female sexual activities were continuously observed to detect the beginning of mating. Once mating occurred and lasted more than 1 min, either no male, one (moderate competition treatment), or three (high competition treatment) rival field males of the same age were immediately added in the mating chamber using a small hole previously drilled in the lid (1 cm diameter). To control if mating males can distinguish the sex of the audience in the mating chamber, some of the males were exposed to one or three additional 2-day-old virgin standardized female (instead of males) originating from the stock population. Once mating finished, the females engaged in pair were collected.

Behavioral and life history traits measurements

Mating behaviors. For the first experiment (male–male competition prior to mating), we recorded the mating latency (time elapsed from the session's start until

Table 1 Effects of the number of conspecific males (0, 1, or 3) and the mass of the two partners engaged in mating on the reproductive traits and behaviors when focal males were exposed to the conspecifics 24 h prior to mating (experiment 1).

	Number of conspecific males		Mass of the male		Mass of the female	
	Test value	*P*	Test value	*P*	Test value	*P*
Mating latency[†]	$\mathbf{F_{2,145} = 14.03}$	**<0.001**	$F_{1,145} = 0.01$	0.94	$F_{1,145} = 0.1$	0.76
Mating duration[†]	$\mathbf{F_{2,148} = 6.28}$	**0.002**	$F_{1,148} = 2.79$	0.1	$F_{1,148} = 0.1$	0.28
Spermatophore volume[†]	$\mathbf{F_{2,75} = 5.59}$	**0.005**	$\mathbf{F_{1,75} = 23.21}$	**<0.001**	$F_{1,75} = 2.83$	0.1
Fecundity[†]	$F_{2,68} = 2.32$	0.11	$F_{1,68} = 0.69$	0.41	$\mathbf{F_{1,68} = 35.16}$	**<0.001**
Fertility[‡]	$\chi^2_2 = 0.003$	0.34	$\chi^2_1 = 0$	0.76	$\chi^2_1 = 0$	0.89

[†] ANCOVA.

[‡] GLM with quasi-Poisson errors.

Note: Bold font indicates statistical significance.

genital coupling) and the mating duration (time between the pair formation and separation) of each pair. For the second treatment (male–male competition during mating), we measured the mating duration.

The spermatophore volume. Immediately after the end of mating, half of the mated females in all modalities of the two experiments 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 to measure its size. Estimating spermatophore size by extrapolating its volume is a well-established method used in several studies on moths (Royer & McNeil, 1993; Foster & Ayers, 1996) and in previous works on *L. botrana* (Torres-Vila *et al*., 1999; Muller *et al*., 2016). We measured its length *l*, width *w*, and thickness *t* 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 & Boag, 1987).

The female reproductive output. After one successful mating and natural separation of the pair, half of the females were individualized in glass tubes (70 × 9 mm diameter) stoppered with moistened cotton plugs, and then stored at 22°C under natural photoperiod. These females were allowed to oviposit freely on the surface of the glass tub until their death. Female survival was checked daily. After the female's death, the eggs were incubated under the same conditions as moth maintenance for seven days. We recorded the achieved fecundity (mean number of eggs laid per female), and the female fertility (proportion of hatched eggs).

Statistical analysis. All the measured traits (mating behaviors, spermatophore volume, and female reproductive output) were studied with linear models after applying square root or log transformations if necessary (mating latency and mating duration) or with GLM-quasi Poisson errors (fertility). For the first experiment (male–male competition prior to mating), the number of conspecific males exposed to the focal male prior to mating, as well as the mass of the male and the female (measured at the pupal stage) engaged in mating were included in the model as independent factor and covariates. For the second experiment (male–male competition during mating), models incorporated the number of conspecifics exposed to the focal male during mating, their sex, and their interaction as factors. The mass of the males and the females engaged in mating were also included in the model as covariates. All these data were studied with analyses of covariance (ANCOVAs). Analyses that revealed significant effects were followed by Tukey's post hoc paired comparisons. Parametric assumptions were ascertained through Shapiro–Wilk (normality) and Levene tests (homoscedasticity) conducted on the residuals of the fitted model. All statistical analyses were carried out using R 4.0.5 software (R Core Team, 2021) with core functions and those included in packages ggplot2 (Wickham, 2016), lsmeans (Lenth, 2016), Rmisc (Hope, 2013), and cowplot (Wilke, 2020).

Results

Experiment 1: male–male competition prior to mating

The number of conspecific males exposed to the focal male 24 h prior to mating influenced both the mating latency and the mating duration (Table 1): the higher the number of conspecific males, the shorter the mating latency of the focal male (Fig. 1A) and the

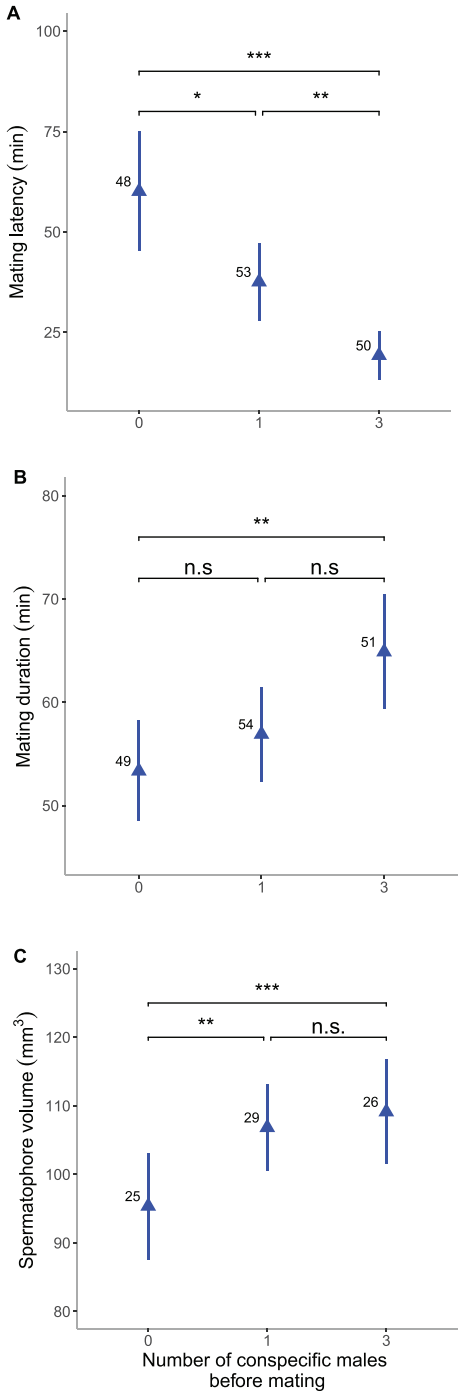

Fig. 1 Effects of the number of conspecific males (0, 1, or 3) on (A) mating latency (time elapsed from the session's start until genital coupling), (B) mating duration (time between the pair formation and separation), and (C) spermatophore volume, in the case where the focal males have been exposed to them 24 h prior to mating. Represented values correspond to means ± 95% confidence intervals. Asterisks highlight significant differences (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, n.s., nonsignificant) and numbers refer to sample sizes.

longer the mating duration (Fig. 1B). The number of conspecific males exposed to the focal male 24 h prior to mating also influenced the spermatophore volume transfer by the focal male to the female (Table 1): the spermatophore volume increased when the focal male was exposed to conspecific males prior to mating (Fig. 1C). It was also influenced by the mass of the male (Table 1), as the spermatophore volume was positively correlated with the mass of the male engaged in mating (Pearson's $r = 0.28$, $t = 2.63$, df = 78, $P = 0.01$). After mating, neither the total number of eggs laid by the females (fecundity), nor the proportion of hatching eggs (fertility) were influenced by the number of conspecific males exposed to the focal male 24 h prior to mating (Table 1). Fecundity was the only trait positively correlated with the mass of the females (Pearson's $r = 0.58$, $t = 5.96$, df = 71, $P < 0.001$).

Experiment 2: male–male competition during mating

The mating duration was influenced by the number of conspecifics exposed to the focal male during mating; this effect depended on the sex of the audience (Table 2): the mating duration shortened only when the focal males was exposed to females (either one or three) during mating (Fig. 2A). Both the number of conspecifics exposed to the focal male and the sex of the audience influenced the spermatophore volume (Table 2): the focal male transferred a larger spermatophore when mating in the presence of one conspecific in comparison with no audience (Fig. 2B). This effect was sex specific as larger spermatophores were transferred only in the case of a male sex audience (Fig. 2C). To note, the spermatophore volume was influenced by the male mass engaged in mating (Table 2), as larger spermatophores were transferred by heavier males (Pearson's $r = 0.32$, $t = 3.75$, df = 122, $P < 0.001$). Regarding the reproductive output of the mated female, neither the total number of eggs laid (fecundity) nor the proportion of hatching eggs (fertility) were influenced by the number of conspecifics exposed to the focal male during mating, whatever their sex (Table 2). As for the first competition experiment, fecundity was the only trait positively correlated with the mass of the females (Pearson's $r = 0.39$, $t = 4.41$, df = 109, $P < 0.001$).

Discussion

The aim of our study was to quantify the ability of Lepidopteran males producing spermatophores to respond and adjust to male–male competition. Sexual audience consisted in three different densities of potential rivals exposed to focal males 24 h before mating or during

Table 2 Effects of the number of conspecifics (0, 1, or 3), the sex of the audience added in mating box (male or female), their interaction, and the mass of the two partners engaged in mating on the reproductive traits and behaviors when focal males were exposed to the conspecifics during mating (experiment 2).

	Number of conspecifics		Sex of the conspecifics		Interaction Number:Sex		Mass of the male		Mass of the female	
	Test value	*P*	Test value	*P*	Test value	*P*	Test value	*P*	Test value	*P*
Mating duration[†]	$\boldsymbol{F_{2,236} = 5.06}$	**0.007**	$F_{1,236} = 3.08$	0.08	$\boldsymbol{F_{2,236} = 3.33}$	**0.037**	$F_{1,236} = 0.15$	0.69	$F_{1,236} = 1.87$	0.17
Spermatophore volume[†]	$\boldsymbol{F_{2,116} = 3.84}$	**0.02**	$\boldsymbol{F_{1,116} = 6.69}$	**0.01**	$F_{2,116} = 1.77$	0.17	$\boldsymbol{F_{1,116} = 20.4}$	**>0.001**	$F_{1,116} = 0.94$	0.33
Fecundity[†]	$F_{2,103} = 1.53$	0.22	$F_{1,103} = 2.30$	0.13	$F_{2,103} = 0.39$	0.68	$F_{1,103} = 0.06$	0.81	$\boldsymbol{F_{1,103} = 15.11}$	**>0.001**
Fertility[‡]	$\chi^2_2 = 0.008$	0.39	$\chi^2_1 = 0.005$	0.29	$\chi^2_2 = 0$	0.93	$\chi^2_1 = 0.003$	0.39	$\chi^2_1 = 0$	0.69

[†] ANCOVA.

[‡] GLM with quasi-Poisson errors.

Note: Bold font indicates statistical significance.

mating. Our results showed that the sexual audience strongly influenced the mating behaviors expressed by the males and the volume of the spermatophore they transferred to the females, but the effects differed according to the time at which the sexual audience is perceived. The mating latency decreased while the mating duration increased when the males were exposed to a larger number of competitors 24 h before mating. During mating, the effect of the sexual audience depended on the sex of the audience as the mating duration decreased and the spermatophore size increased when the focal males were exposed to females. Despite these clear effects of the sexual audience on the male mating behaviors, we did not find any consequences for the reproductive output of the females mated by males experiencing different sexual audiences. Our results showed that males adjust their mating behaviors to a potentially elevated risk of sperm competition, but the various behavioral (i.e., mating latency and mating duration) and physiological responses (i.e., spermatophore size) are not fully reflected in their reproductive performance.

Males exposed to different sexual audiences before and during mating expressed plastic sexual behavior that are consistent with risk model predictions (Parker *et al*., 1997; Engqvist & Reinhold, 2005). They showed higher motivation to access the available females and invest more during mating through longer mating duration when having exposed to potential rival males before mating. Previous studies in various species have reported extended mating duration when males were exposed to competitors prior to mating (Bretman *et al*., 2009, 2013; Price *et al*., 2012). Mating duration is known as a plastic trait highly responsive to the sociosexual context during which it is expressed (Bretman *et al*., 2011a, 2013). A reduced mating latency associated with a prolonged mating duration may generate at least two benefits for the male, that would be verified with similar experiments: to have a privileged access to the females by outperforming the mating ability of the other males, and to decrease the propensity of the mated females to remate subsequently. Faced with this significant risk emerging when multiple males look for females, extended mating duration is viewed as a "mate guarding strategy," which significantly decreases sperm competition intensity in the female genital tract (Carazo *et al*., 2007; Mazzi *et al*., 2009). In our study system, the extended mating duration likely initiated by the male seems beneficial for him as it may prevent females from remating immediately after the pair separation, ensuring the transferred sperm to fertilize the eggs (Torres-Vila *et al*., 1997; Gilchrist & Partridge, 2000; Muller *et al*., 2016). Remaining in pair for more than 1 h is sufficient to reduce the probability that a female remates on the same day, mating occurring *in natura* at dusk (Louâpre and Moreau, personal observation). Moreover, sperm generally reaches the spermatheca between 2 and 5 h after mating in several butterfly and moth species (Seth *et al*., 2002; Marcotte *et al*., 2005). The plastic behavior expressed by males experiencing male–male competition prior to mating could be particularly efficient in *L. botrana*, but it is usually observed in species with short mating or external spermatophore transfer (Simmons, 2002).

Plasticity in the male reproductive investment and mating behaviors is known to evolve rapidly in populations depending on the sexual audience males are faced with (Dore *et al*., 2021). Here, such plasticity in mating

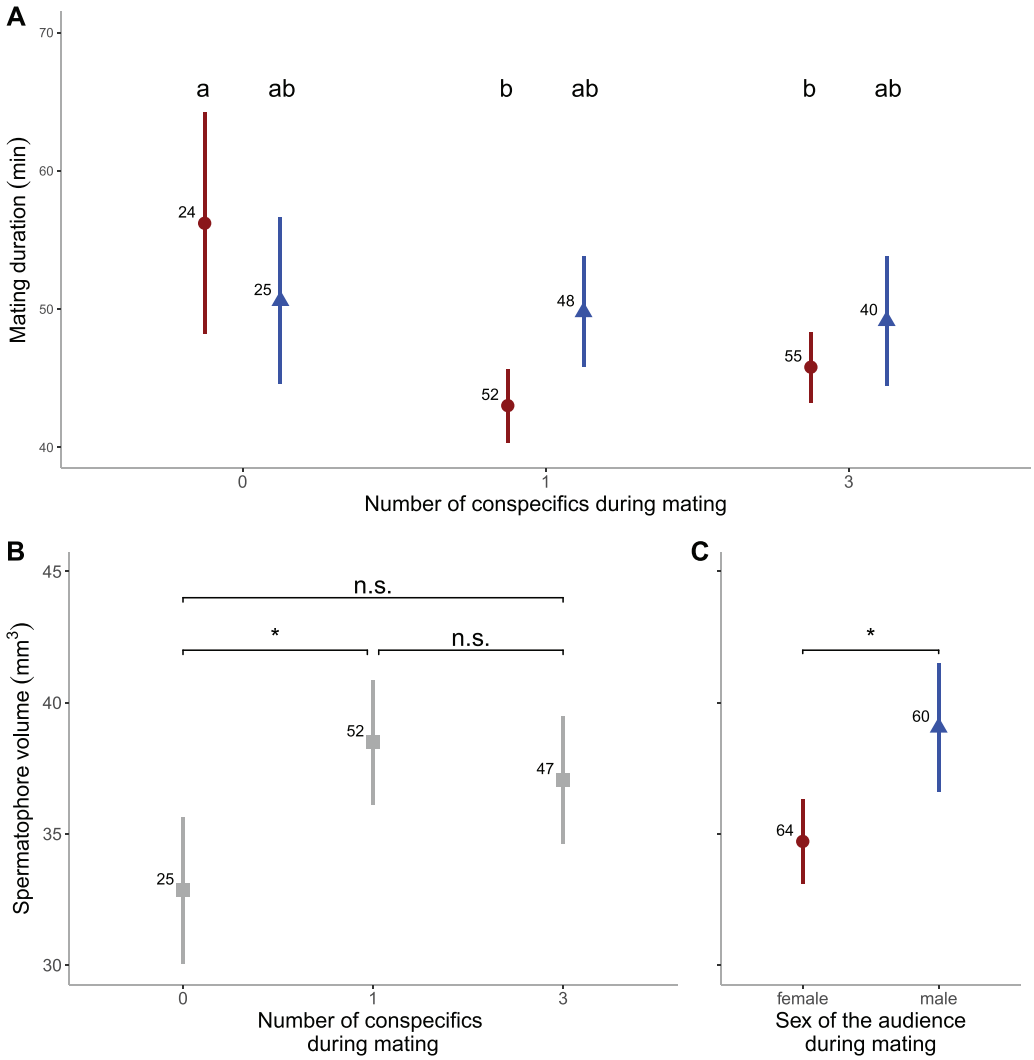

Fig. 2 Effects of the number of conspecifics (0, 1, or 3) and their sex (males are represented by triangles, females by points, squares represent the combined effect of the two sexes) on (A) mating duration (time elapsed from the session's start until genital coupling), (B and C) spermatophore volume, in the case where the focal males are exposed to a sexual audience when mating. Represented values correspond to means ± 95% confidence intervals. Letters and asterisks highlight significant differences (*$P < 0.05$, n.s., nonsignificant) and numbers refer to sample sizes.

behaviors is only expressed when males perceived potential rival males 24 h before mating. We could not find any effect of the presence of rival males on the mating duration when they were perceived by the focal male during mating. Our results thus show that the sexual audience is a key information perceived and retained by the insect males for at least 24 h, and responsible for the expression of subsequent plastic mating behaviors, even when potential rivals disappear at the moment of mating. Rouse *et al*. (2018) demonstrated that a plastic response of *Drosophila melanogaster* males to sperm competition is based on their ability to assess the sexual audience when exposed to rival males through olfactory learning and memory. Long-lasting memory of sperm competition risk experienced by males is suspected to be involved to assess the sociosexual context through various cues (acoustic, chemical, visual, tactile), as it has been shown in *D. melanogaster* (Bretman *et al*., 2011b) and the seed beetle *Callosobruchus maculatus* (Liu *et al*., 2020). A similar cognitive ability might guide the expression of plastic mating behaviors in *L. botrana* males: one can speculate that the presence and/or the number of rival males may be information retained by the nervous system causing later arousal of the male's motivation

faced with females. However, males can perceive the sexual audience during mating if composed of females, as in our experiments, the mating duration decreased when supplementary females were in closed proximity of the pair. Such a sex-specific effect, presumably caused by detecting further opportunity of mating by the male, reinforces the hypothesis that multiple cues are perceived by males engaged in mating to assess the quality and the density of the sexual audience.

Besides plastic mating behaviors expressed by males responding to the sexual context before and during mating, they also exhibit a plastic physiological response. They transferred a bigger spermatophore when exposed to conspecifics; this effect was observed within the two competition experiments when males were exposed to competitors 24 h before mating, or during mating. We thus highlight the ability of *L. botrana* males to express a physiological response quickly depending on the perceived sexual context, which may improve its fitness relative to competitors. When polyandry occurs in *L. botrana*, the likelihood for the female to remate strongly depends on the spermatophore size it received by the first male: the bigger the spermatophore transferred, the lower the propensity of remating (Torres-Vila *et al*., 2002). The higher investment by the male thus assures a higher paternity under male–male competition. In a previous study on *L. botrana*, it has been shown that a bigger spermatophore (for which a significant part of variance was attributed to the host plant consumed by the males at larval stages) induced higher fecundity (Muller *et al*., 2015). Higher investment in the male's ejaculate—in this case, after exposure to rival males—translates into an increase of its reproductive success, as it was also the case for example in *D. melanogaster* (Bretman *et al*., 2009) and the beetle, *Tenebrio molitor* (Gage & Baker, 1991). Here, such plastic response was not followed by an increase in fecundity or fertility by the mated female. Regarding our results, two plausible explanations of these diverging results may emerge from the reproduction mode of moths. First, the lack of the expected fitness gain for *L. botrana* males faced with rival males suggests that the bigger spermatophores transferred by the male result from higher investment in nonsperm components rather than on sperm allocation. Indeed, insect males produce internal spermatophores containing sperm and various secretions produced by accessory glands, which modulate mating behaviors and reproductive output of the females (Gillott, 2003; Perry *et al*., 2013; Ramm, 2020). Second, males *L. botrana* exposed to rivals may also modify their investment in the two sperm forms (i.e., a fertile eupyrene form and a nonfertile apyrene form), by increasing the production of apyrene sperm (Silberglied *et al*., 1984; Gage & Cook, 1994; Swallow & Wilkinson, 2002). Such an adaptive strategy may have no direct consequence on the female fecundity and fertility, but it may impact the mating behaviors of females, especially their propensity for remating. Hypothetic mechanisms behind adjustment of sperm quality and quantity mostly involve changes occurring during sperm maturation, which require at least several days (Magris, 2021). Consequently, during our experiments lasting from 24 h prior mating to the moment of mating, rapid changes in spermatophore composition in response to the sexual audience are more likely to be mediated by adjustment of nonsperm component rather than by sperm modification.

To conclude, this study clearly shows that males are highly sensitive to the sexual audience before and during mating and can adapt their mating behavior and physiological response accordingly. These plastic responses are expected to evolve in situations where the probability to find a mate is stochastic in a short temporal window. In many lepidopteran species, encountering a female is rare and the first mating induces a strong inhibition of female mating (Parker & Vahed, 2010; Jarrige *et al*., 2016). The selective pressure exerted on males experiencing male–male competition could be sufficient to trigger adjustment in male mating behavior according to the perception of competition and to select sensitive mechanisms allowing to perceive competition.

Disclosure

The authors declare they have no conflict of interest.

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