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Behavioral Ecology

Lateralized courtship behavior in *Ostrinia furnacalis* (Lepidoptera: Crambidae): influence of gender, sexual experience, and its effects on mating success

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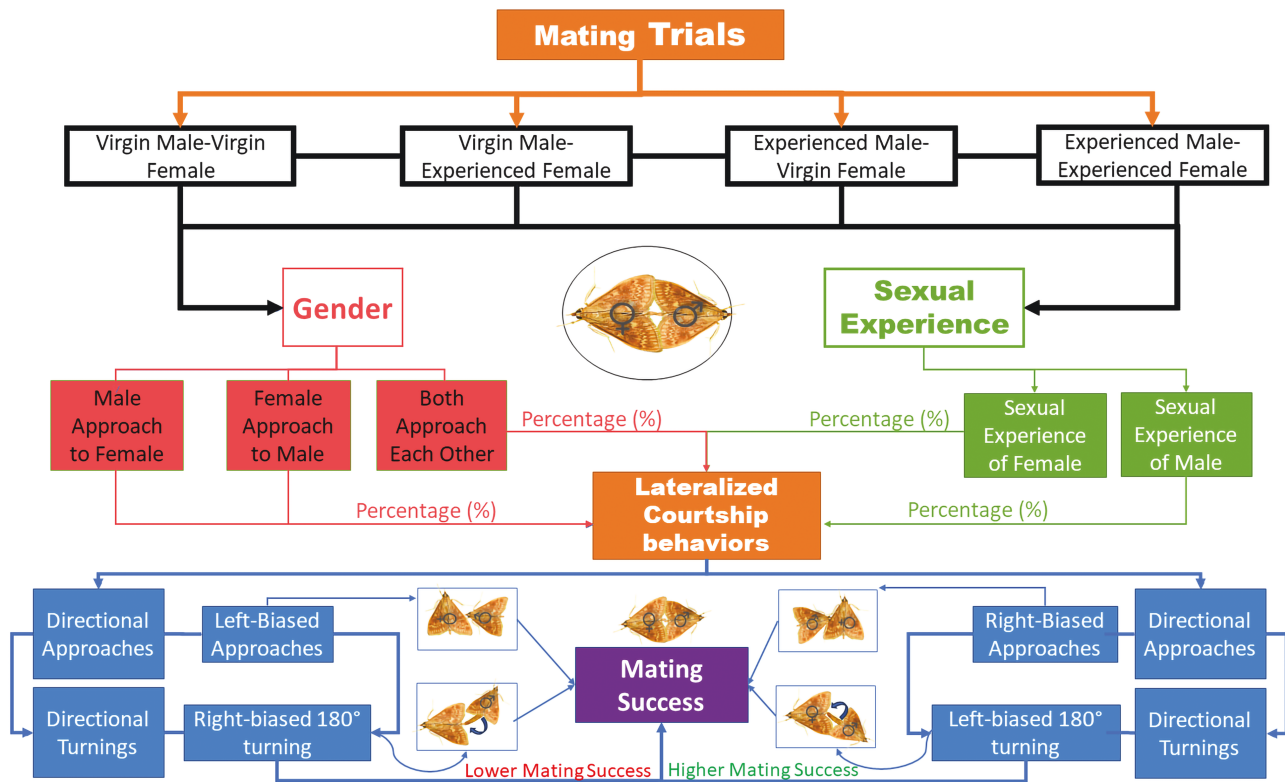
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Lateralization in mating behavior is increasingly recognized as a significant trait in insect species, yet its influence associated with gender, and sexual experience in *Ostrinia furnacalis* (Guenée) remains poorly understood. This study examines how lateralized mating behaviors, gender, and sexual experience interact to influence mating success and efficiency in *O. furnacalis*. We conducted controlled mating trials to assess how gender and sexual experience shape lateralized directional approaches (eg right- or left-biased) and turnings (eg 180° right- or left-biased) across the pre-copulatory, copulatory, and post-copulatory phases. Our results indicated that, in terms of gender, males were more likely to approach females, whereas females rarely initiated approaches, with both approaches each other simultaneously being infrequent. Both virgin and experienced males showed higher right-biased directional approaches than the front approaches to the females with more left-biased directional turns for successful intromissive copulation. Experienced males showed greater mating success than virgins. In contrast, experienced females exhibited lower mating success and longer post-copulatory interactions compared to virgin females, particularly duration of copulation. Post-copulatory interactions showed that antennal touching occurred more frequently in the experienced pairs. This study is the first to demonstrate the combined influence of gender and sexual experience on lateralized mating dynamics, with male courtship behaviors linked to learning processes. The results indicate that sexual experience, potentially involving learning and memory processes, significantly enhances mating efficiency and fitness in *O. furnacalis*. This research provides a more nuanced understanding of lateralized mating behaviors in *O. furnacalis*, with implications for refining pest management strategies in agricultural environments.

Keywords: *O. furnacalis*, lateralization, courtship behavior, sexual experience, insect neurology, mating laterality

Graphical Abstract



Introduction

Lateralization, the asymmetrical organization of brain functions and behaviors between the left and right sides in animals and insects, has long intrigued neuroscientists (Güntürkün et al. 2020, Rogers 2021, Donati et al. 2024, Ocklenburg and Guo 2024). This specialization of brain function often results in a preference or dominance in movement directionality during the development of certain behaviors, leading to asymmetric actions such as dominance in the use of the right or left hand in humans (Labache et al. 2023, Rogers 2024). Laterality exists at 2 levels: the individual level, where an individual shows a preference for one side, and the population level, where most individuals in the group consistently display the same directional biases (Vallortigara 2006, Frasnelli and Vallortigara 2018). In insects, lateralization is observed during various activities, but it becomes particularly pronounced during mating phases (ie approaching and mounting) when adults perform lateralized movements (ie to the right or left-biased). These differences in directionality can affect mating success, offering significant functional advantages that contribute to biological fitness (Vallortigara and Rogers 2005, Schilthuisen 2007, Winding et al. 2023). Although diverse insects have shown preferences for one side of the mate's body or a tendency to turn in a particular direction during mating (ie mating behavioral asymmetries; Frasnelli et al. 2012b, Rogers et al. 2013, Schilthuisen 2013, Soma 2022), particular attention has been focused on pest species (Kamiya et al. 2015, Romano et al. 2016, 2020, Benelli et al. 2017a, Boukouvala et al. 2020, 2021) as understanding these behaviors may shed light on their reproductive success.

Premating and mating behaviors in insects are influenced by gender and sexual experience (Benelli et al. 2017b, Boukouvala et al. 2021, Calla-Quispe et al. 2022), which affect mating success. In

this context, lateralized behavior during the directional approaching and turnings for intromissive copulation of the mating pairs may confer advantages in increasing the efficiency, accuracy, or speed in mating, while non-lateralized behavior may allow for quicker and more flexible responses to changing mating conditions (Frasnelli 2017, Frasnelli and Vallortigara 2018, Álvarez-Quintero et al. 2021, Knebel and Rigosi 2021). In pest species such as *Cryptolestes ferrugineus*, *Tribolium castaneum*, and *Trogoderma granarium*, males with left-biased approaches have been shown to exhibit higher mating success compared to those with right-biased or non-lateralized behaviors (Kamiya et al. 2015, Romano et al. 2016, 2020, Benelli et al. 2017a, 2017b, Boukouvala et al. 2022). The effectiveness of these lateralized behaviors can vary based on gender and sexual experience, emphasizing their role in the mating system and the overall reproductive strategy of some pests (Romano et al. 2016, Boukouvala et al. 2020, 2021, Anton and Rössler 2021). Therefore, understanding how gender and sexual experience interact with lateralized courtship and mating behaviors is essential for elucidating their effects on mating success.

The Asian corn borer (*Ostrinia furnacalis* Guenée; Lepidoptera: Crambidae), a significant polyphagous pest, poses a major threat to maize crops across Asia and Europe (Chen et al. 2017, Alam et al. 2024, Li et al. 2024). Understanding its intricate lateralized mating behaviors based on gender and sexual experience is crucial for developing effective pest management strategies. While previous research has suggested that factors such as lateralization, gender, and sexual experience influence mating success and efficiency in *Alphitobius diaperinus* (Calla-Quispe et al. 2023), the specific interactions among these factors in *O. furnacalis* have not been thoroughly investigated. Lateralized behaviors, including directional approaches and turnings, play a significant role in mating success for *O. furnacalis* (Abbas et al. 2024) but the combined impact of

gender and sexual experience on lateralized courtship behaviors and mating success in *O. furnacalis* has not been thoroughly explored. Furthermore, understanding how lateralized behaviors influence the entire mating sequence—including pre-copulatory displays, the copulatory phase, the post-copulatory phase, mating success, and the efficiency of intromissive copulation—remains essential for enhancing our knowledge of these interactions. Since experienced males may exhibit more distinct and efficient behaviors (ie shorter pre-mating and mating sequences leading to copulation) compared to virgin males, potential behavioral asymmetries (ie lateralized and non-lateralized movements) may be linked to changes in sexual experience and gender (Calla-Quispe et al. 2023). However, whether these differences occur in *O. furnacalis* has not been previously investigated. This study aims to investigate the occurrence and role of lateralized and non-lateralized mating behaviors on mating success and efficiency, as well as their association with gender and sexual experience in *O. furnacalis*. To achieve this, we conducted mating behavioral trials with adult moths, measured the magnitude of lateralized behaviors, and depicted them in an ethogram. Statistical tests were performed to determine how these factors influenced mating outcomes. Importantly, our results provide the first report demonstrating how gender and sexual experience jointly influence mating dynamics in *O. furnacalis*, emphasizing the role of behavioral asymmetries and learning in enhancing reproductive fitness. By elucidating the relationships between lateralization, gender, and sexual experience in the mating behaviors of *O. furnacalis*, this study provides valuable insights into its reproductive ecology and behavioral dynamics. These findings enhance our understanding of the neural and ecological mechanisms underlying mating success in this species, contributing to broader research on insect behavioral neurobiology and reproductive strategies.

Materials and Method

Studied Species and Cultures in the Laboratory

Adults were sourced from a previously established laboratory culture at the College of Plant Protection, Jilin Agricultural University, as described in our previous study on the exploration of lateralization in *O. furnacalis* (Abbas et al. 2024). Gender identification was conducted during the adult phase, categorizing individuals as male (denoted as “M”) and female (denoted as “F”) based on their morphological characteristics (Mutuura and Munroe 1970). Newly emerged male and female adults were placed in mesh cloth cages (30 cm diameter × 30 cm height) to facilitate mating and reproduction, resulting in experienced individuals (denoted as “exp”). In contrast, other newly emerged adults were kept in plastic round cups with lids (3.5 cm diameter × 3.5 cm height) that had small holes to ensure proper air circulation and prevent excessive moisture accumulation. To ensure proper nourishment, all adults were provided with a 10% honey solution on cotton bolls, which was replenished daily. These individuals remained without sexual contact and were classified as virgins (denoted as “v”). All these bioassays were conducted under controlled laboratory conditions at a temperature of 28 ± 2 °C and relative humidity (RH) of 60 ± 5 % to ensure consistent environmental parameters (Calla-Quispe et al. 2023). The creation of both experienced and virgin groups was essential to investigate the impact of sexual experience on lateralized mating behaviors in *O. furnacalis*.

Experimental Groups

The mating behavior experiments involved 4 groups based on gender and sexual experience: Mv–Fv (male virgin and female

virgin), Mv–Fexp (male virgin and female experienced), Mexp–Fv (male experienced and female virgin), and Mexp–Fexp (male experienced and female experienced). All individuals in these bioassays were no more than 1 d old, and each was used only once.

Experimental Setup

Newly emerged males and females (within 10 h of emergence) were either allowed to mate, becoming “experienced,” or kept as virgins for the experiments. Experienced adult moths were given a 10–15 h recovery period after mating before being used in bioassays. Virgin and experienced adult moths were consistent in age between groups. This timing protocol was designed to eliminate potential age-related effects on mating behavior. Before behavioral testing, adults were acclimated for 5 to 10 min. Acclimation ensured the insects adjusted to the environment before testing. Mating behaviors were observed in experimental mesh cloth cages (30 cm diameter × 30 cm height), lined with filter paper to reduce visual stimuli that might influence behavior and proper diet (Romano et al. 2016).

Behavioral Observations

Mating behaviors were recorded in a sequence from precopulatory to postcopulatory phases (see ethogram in Fig. 1A). Males and female moths were placed at opposite ends of the cage and their interactions were observed visually (Observer: Abbas). The following behavioral elements were documented:

- a. **Gender Approach and Male Directional Approaches:** The sex that initiated the approach (male, female, or both) and the direction of the male’s approach (right-biased [Ra], left-biased [La], or frontside [Fa]; Fig. 1B).
- b. **Male Directional Turnings:** The direction of the male’s turning during intromissive copulation (right-biased 180-degree turning [Rbt], left-biased 180-degree turning [Lbt], or frontside approach [Fa]; Fig. 1C).
- c. **Mating Phases:**
 - **Mate Recognition:** The time a male spends recognizing a female before mating initiation.
 - **Male Ultrasonic Courtship:** Duration of male wing fanning until intromission.
 - **Antennal Contact:** Time the male spent contacting the female’s posterior with his antennae.
 - **Intromissive Copulation:** Duration from genital insertion to the completion of copulation.
 - **Post-copula Interaction:** Time spent in post-copulatory contact, including antennal contact.

The number of successful and unsuccessful mating attempts was also recorded for each trial.

Data Collection and Statistical Analysis

A total of 238 trials were conducted, of which 24 were excluded due to the absence of behavioral interactions (ie no approach, precopulatory, or copulatory behaviors). Thus, 214 trials were included in the analysis, with the following sample sizes: Mv–Fv ($n = 50$), Mv–Fexp ($n = 57$), Mexp–Fv ($n = 50$), and Mexp–Fexp ($n = 57$). We conducted a power analysis before data collection, which indicated that the sample size was sufficient to detect the expected effects with a statistical power of 0.8 and a significance level of 0.05. The power analysis, considering 4 groups (Mv–Fv, Mv–Fexp, Mexp–Fv, Mexp–Fexp), a medium effect size (Cohen’s $f = 0.25$), and 80% power, suggested a required sample size of approximately 30 subjects per group (Torres-Vila and Jennions 2005).

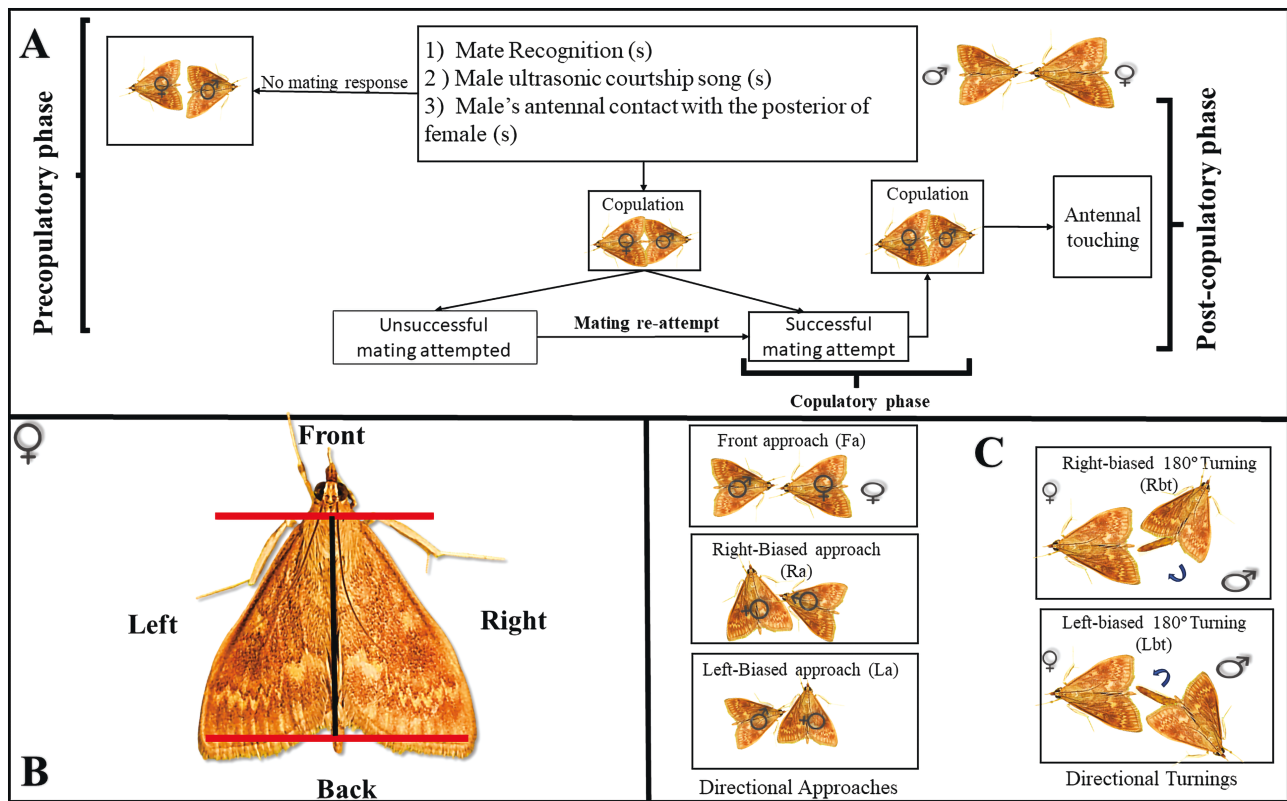


Fig. 1. Ethogram depicting (A) the sequence of mating behavior, (B) graphical representation of the directional approaches by the mate, and (C) directional turnings for intromissive copulation (right-biased 180-degree turnings [Rbt], left-biased 180-degree turnings [Lbt], or frontside [Fa] approaches relative to the female) in *Ostrinia furnacalis*.

Given this, our sample sizes were adequate to reliably detect significant differences in mating behavior across 4 groups. For the quantitative analysis, each trial was treated as a single observational unit, regardless of the number of mating attempts. This approach ensured that the entire behavioral sequence (pre-copulation, copulation, and post-copulation phases) was analyzed as one comprehensive event, minimizing potential bias from repeated mating attempts within a single trial (Calla-Quispe et al. 2023).

To test for differences in which gender performed the approach to its mate, the proportion of individuals initiating the approach was compared using a chi-square (χ^2) test with Yates' correction ($P \leq 0.05$).

To evaluate the effect of *O. furnacalis* gender performing the directional approach and the directional turnings on mating success, we used a generalized linear model with a binomial distribution and a logit link function, as described below:

$$\gamma = MP + IA + DA + \epsilon \quad (1)$$

where γ 1 is the vector of observations (i.e., successful or unsuccessful mating), MP represents the mating pair combination, IA is the gender performing the approach, DA is the direction of the approach, and ϵ 1 is the vector of random residual effects.

To assess the effect of male intromissive copulation during directional turnings on mating success, we used another generalized linear model with a binomial distribution and a logit link function:

$$\gamma = MP + DT + \epsilon \quad (2)$$

where γ 2 is the vector of observations (ie successful or unsuccessful mating), MP is the mating pair, DT represents the direction of turning, and ϵ 2 is the vector of random residual effects.

In this case, 3 scenarios were evaluated independently: (i) when the approach was initiated by males, (ii) by females, or (iii) by both approached each other simultaneously.

To examine the effect of the directionalities (directional approach and turnings) during mating behavior on the duration of pre-copula behavioral displays and intromissive copulation, and their association with gender and sexual experience, we implemented 2 log-truncated linear models. One model used the duration of pre-copula behavioral displays as the response variable, while the other used intromissive copulation duration. The directionalities of mating behavior and mating pair combinations were set as explanatory variables. Bonferroni post hoc tests were then conducted. Both pre-copula and copulation durations were log-transformed before analysis to meet assumptions of normality. Post hoc comparisons using Bonferroni tests were conducted to evaluate the statistical differences between experimental groups. In cases where multiple comparisons were needed, Bonferroni correction was applied.

All statistical analyses were conducted using the stats (Team 2020) and emmeans packages (Lenth et al. 2023) in the R software (version 4.1.2)(Wickham et al. 2023).

Results

Mating Behavior Sequence

In the precopulatory phase, either or both members of the male-female pair initiate lateralized behavior (directional approaches and turning from the right or left-biased) or non-lateralized behavior (approaching from the front) toward their mate. Upon approaching, males make physical contact with the posterior and frontal sides of

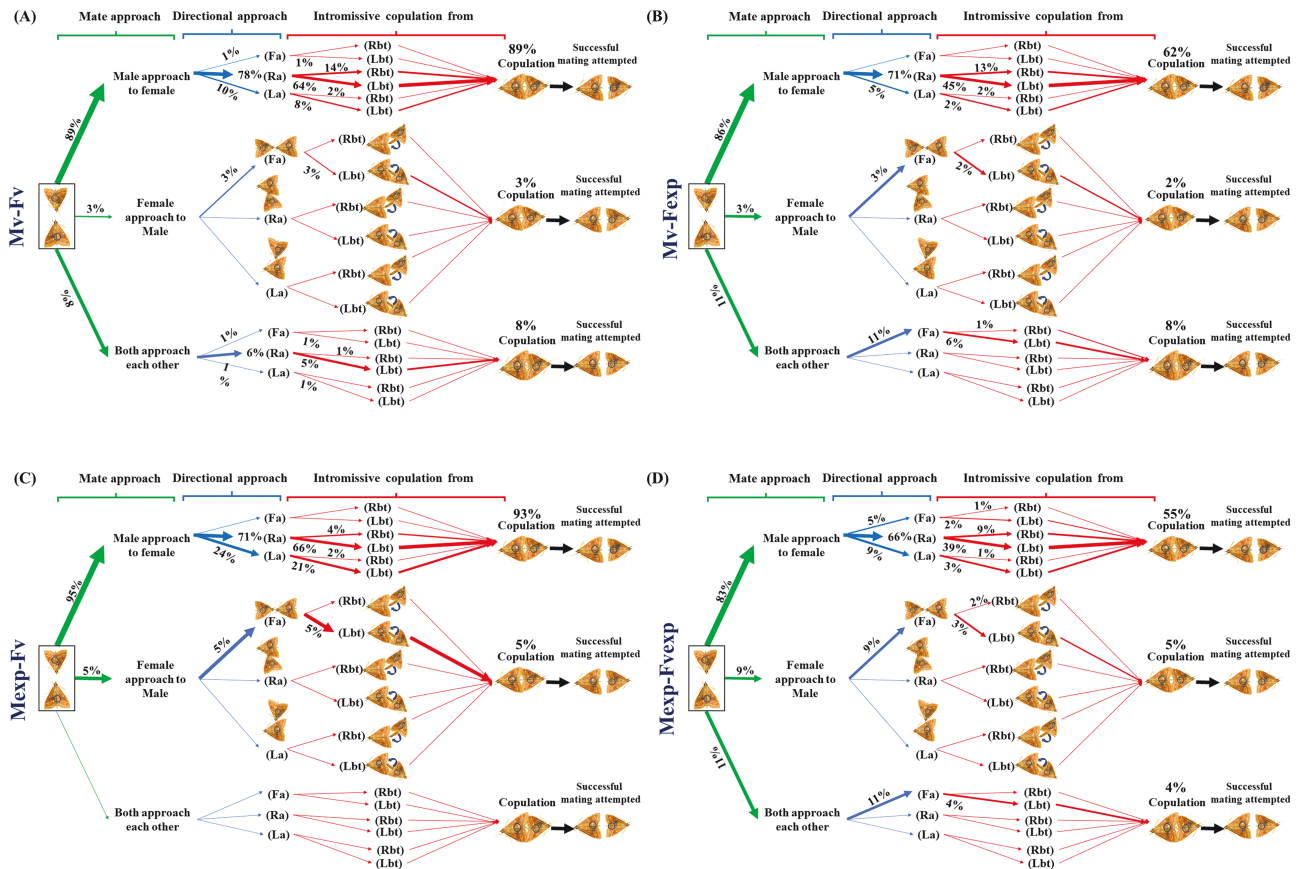


Fig. 2. Flow chart quantifying the proportion of directional approaches (%) and intromissive copulation by directional turnings (%) among the pair mating combinations of *Ostrinia furnacalis*: (A) Mv-Fv, (B) Mv-Fexp, (C) Mexp-Fv, (D) Mexp-Fexp. The thickness of the arrows represents the proportion of adults exhibiting each behavior during both the approach and directional turnings for the success (%) of the mating sequence.

the female using their antennae. Out of all the observed pairs, only 170 male–female pairs exhibited mating behaviors, and these were included in the analysis. When a male encounters a female, a distinct behavioral sequence unfolds. Initially, the male engages in mate recognition, which plays a critical role in the progression of courtship. Upon successful recognition, the male initiates ultrasonic courtship songs by vibrating his wings while gently tapping the female's body with his antennae. As the courtship progresses, the male approaches the female's abdomen and delicately touches its posterior with his antennae. This tactile interaction often prompts the female to raise her abdomen, signaling her receptivity. At this point, the male exhibits directional turning behavior by rotating his body either left or right, performing a 180-degree to align himself for intromissive copulation (Fig. 1A–C). In the later stages of courtship, the female typically becomes motionless, which serves as a crucial indicator of her readiness for copulation. This immobility signals the beginning of the transition to genital alignment and end-to-end contact, which is essential for copulation. In the postcopulatory phase, after mating has concluded, both adults engage in antennal contact, touching each other's head, pronotum, and elytra. This postcopulatory interaction appears to be part of a closing sequence following mating when both adults are touching each other with their antennae (Fig. 1A). Out of 214 adult pairs who exhibited pre-copulatory mating behaviors, including approaches, only 170 resulted in successful intromissive copulation. The remaining trials, despite displaying mating behaviors, failed to achieve mating success (intromissive copulation) and were thus classified as mating unsuccessful.

Effect of Lateralized Mating Displays Associated with Gender and Sexual Experience on Mating Success

The approaching behavior of *O. furnacalis* mating pairs significantly varied based on the gender of the moth initiating the approach ($\chi^2 = 50.9$, $df = 6$, $P < 0.0001$). Males predominantly initiated the approach in 89%, 86%, 95%, and 83% of the interactions in the Mv-Fv, Mv-Fexp, Mexp-Fv, and Mexp-Fexp groups, respectively (Fig. 2). In the combinations involving virgin males (Mv)—such as Mv-Fv and Mv-Fexp—males displayed lateralized behavior, predominantly approaching from the right side of the female (71% and 78% in Mv-Fv and Mv-Fexp, respectively, Fig. 2). In contrast, males in the Mexp also showed higher lateralized approaches compared to Mv, but with lower frequency when paired with Fexp (71% and 66% in Mexp-Fv and Mexp-Fexp; Mv-Fexp and respectively, Fig. 2).

Female approaches were uncommon; however, when they did occur, they were predominantly front-side approaches. Notably, only Fexp displayed non-lateralized behaviors (Fig. 2). Although most non-lateralized approaches resulted in mating, lateralized approaches proved universally successful, leading to copulation in every case (Fig. 2). Interestingly, simultaneous approaches by both genders were rare but more frequent than female-only approaches (Fig. 2). After the approach, a higher proportion of Mv and Mexp proceeded to intromissive copulation through left-biased 180-degree turnings (Lbt) from the female, as compared to right-biased turnings (Rbt). Left-biased turning (Lbt) intromissive copulation occurred in

64%, 46%, 66%, and 30% of cases in the Mv–Fv, Mv–Fexp, Mexp–Fv, and Mexp–Fexp groups, respectively (Fig. 2).

Pair combinations without 2 experienced adults (ie Mexp–Fexp) consistently showed a higher frequency of left-biased 180-degree turns (Lbt) compared to right-biased turns (Rbt) during intromissive copulation. These rates were 64%, 45%, and 39% for Mv–Fv, Mv–Fexp, and Mexp–Fv pairs, respectively (Fig. 3). Additionally, lateralized behaviors predominated over non-lateralized ones in the Mexp–Fexp combinations (Fig. 2). No front-mounted approaches were recorded in mating pairs involving Fv or any combinations including Fv (Fig. 2). Experienced males (Mexp) showed a higher success rate of intromissive copulation than Mv, all intromissive copulations performed by Mexp resulted in successful mating, whereas this was not always the case for Mv (Fig. 2). Furthermore, Fexp exhibited a higher rate of unsuccessful mating attempts compared to Fv. Despite this, experienced females showed a higher rate of intromissive copulation through left-biased 180-degree turnings (Lbt) compared to other lateralized and non-lateralized behaviors (Fig. 2).

Effect of Directional Approaches and Intromissive Copulation with Directional Turnings on Mating Success

The directional approach between adults did not significantly influence overall mating success ($\chi^2 = 0.27$, $df = 210$, $P = 0.8844$). However, the right-biased approach was significantly ($\chi^2 = 14.6$, $df = 210$, $P = 0.0048$) more successful than the left-biased approach. Mating success varied based on the combination of adults (virgin or experienced). Specifically, the mating success in the Mexp–Fexp group was significantly lower than in the Mexp–Fv group ($\chi^2 = 13.9$, $df = 210$, $P = 0.0053$). Significant differences were also observed among other pair combinations, with higher success rates in left-biased 180-degree turnings. The combined effect of the approaching gender and turning direction had a significant impact on the mating success ($\chi^2 = 68.1$, $df = 210$, $P < 0.0001$). Furthermore, the interaction between the adult pair type, gender approach, and direction of approach significantly influenced mating success ($\chi^2 = 74.9$, $df = 210$, $P < 0.0001$). When males initiated the approach, mating success was influenced by both the pair combination ($\chi^2 = 12.1$, $df = 210$, $P = 0.0072$) and the direction of intromissive copulation, particularly with left-biased 180-degree turnings ($\chi^2 = 0.1$, $df = 210$, $P = 0.7115$). Specifically, mating success was lower in the Mv–Fexp group compared to other combinations. In contrast, when females initiated the approach, neither the pair combination nor the direction of intromissive copulation had a significant effect on mating success. When both genders approached simultaneously, the direction of intromissive copulation did influence mating success ($\chi^2 = 6.0$, $df = 210$, $P = 0.0141$), with males performing intromissive copulation from left-biased 180-degree turning being more successful than those from right-biased 180-degree turning (Fig. 3).

Effect of the Mating Pair, Directional Approaching, and Intromissive Copulation with Directional Turnings on Interaction Duration

In our experiments, no significant differences were observed across mating pair combinations in the following parameters: time for mate recognition ($F_{1,210} = 0.36$, $P = 0.7495$), male ultrasonic courtship song ($F_{1,210} = 0.17$, $P = 0.8115$), and male antennal contact with the female's posterior ($F_{1,210} = 0.21$, $P = 0.6008$). Similarly, no significant differences were found in copulation ($F_{1,210} = 0.99$, $P = 0.1897$), post-copulation behavior ($F_{1,210} = 1.10$, $P = 0.2978$), or the entire mating sequence ($F_{1,210} = 1.03$, $P = 0.5342$; Table 1). We observed significant effects of

female sexual experience on the duration of intromissive copulation ($F_{1,166} = 4.80$, $P = 0.0312$) and post-copula duration ($F_{1,166} = 5.83$, $P < 0.05$). Specifically, the Mexp–Fexp pair had significantly shorter durations of intromissive copulation (mean \pm SD: 2940 \pm 92.54s) compared to the Mv–Fv pair (mean \pm SD: 4173 \pm 96.80s) (Table 1). Additionally, experienced females exhibited longer post-copula times compared to Fv (Table 1). However, no significant differences were found regarding the sexual experience of males (Intromissive copulation: $F_{1,166} = 1.40$, $P = 0.2411$; Post-copula: $F_{1,166} = 1.40$, $P = 0.2411$; Whole mating duration: $F_{1,166} = 1.40$, $P = 0.2411$), but female experience had a significant impact (Intromissive copulation: $F_{1,166} = 7.80$, $p = 0.4063$; Post-copula: $F_{1,166} = 8.83$, $P = 0.0068$, Table 1). In pairwise comparisons, post-copula duration in Mexp–Fexp was longer (mean \pm SD: 18.3 \pm 19.3s) than in Mv–Fexp (mean \pm SD: 13.3 \pm 25.8s, $P = 0.0297$, Table 1). No significant differences were observed when comparing Mexp–Fexp with Mexp–Fv or Mv–Fv (all $P > 0.05$, Table 1). The combined effect of gender and sexual experience was not significant ($F_{1,166} = 1.00$, 0.2978, Table 1). Additionally, the duration of mate recognition ($F_{2,54} = 6.09$, $P = 0.0500$), the male ultrasonic courtship song duration ($F_{2,54} = 7.11$, $P = 0.0491$), and the male's antennal contact with the female's posterior ($F_{2,54} = 8.41$, $P = 0.0410$) were significantly affected by the directional approaches in the Mexp–Fexp (Table 2). Furthermore, the duration of directional turnings was significantly different ($F_{1,54} = 5.64$, $P = 0.0365$) in the Mexp–Fv pair, but was non-significant in other mating combinations (Table 3).

Discussion

Our study presents a thorough analysis of the interplay between gender and sexual experience in shaping lateralized courtship behaviors and their consequent effects on mating success in *O. furnacalis*. Both lateralized and non-lateralized behaviors during mating, particularly in the directional approach and directional turnings for intromissive copulation, are significantly modulated by these factors, with critical implications for reproductive fitness. Previous research has established that lateralized movements in insect mating are often associated with underlying anatomical asymmetries and the localization of specific glands, which amplify sensory cues during courtship and enhance reproductive outcomes (Anfora et al. 2010, Frasnelli et al. 2012a, Schilthuisen 2013, Knebel and Rigosi 2021). Despite minor morphological asymmetries, the abdomens of beetles appear predominantly symmetrical, with both sexes possessing a secretion reservoir at the ventral apex (Tseng et al. 1971, Rončević et al. 2024). This symmetry may explain the prevalence of non-lateralized behavior during approach and intromissive copulation. In contrast, lateralization is more common among sexually experienced males, suggesting that learning improves mating success (Filice et al. 2021). The tendency for males to approach females from the left implies specialized neural circuits facilitate lateralized sensory-motor responses, enhancing gender recognition (Romano et al. 2020). The varied approaches of experienced adults may result from anatomical changes during the adult phase, similar to findings in other insects (Salin et al. 2000, Budečević et al. 2021). In the postcopulatory phase, experienced females of *O. furnacalis* show a reduced tendency to remate, unlike species such as *Aphidius ervi*, where remating is common (Ode et al. 2022). This decreased remating ability in experienced females (Fexp) may lead to unsuccessful mating attempts for males, prompting them to seek additional opportunities, thus increasing their reproductive success. Postcopulatory mate guarding, often observed after insemination, acts to prevent sperm competition from other males (Elias et al. 2014, Parker 2020, Matzke et al. 2023). While our study has demonstrated

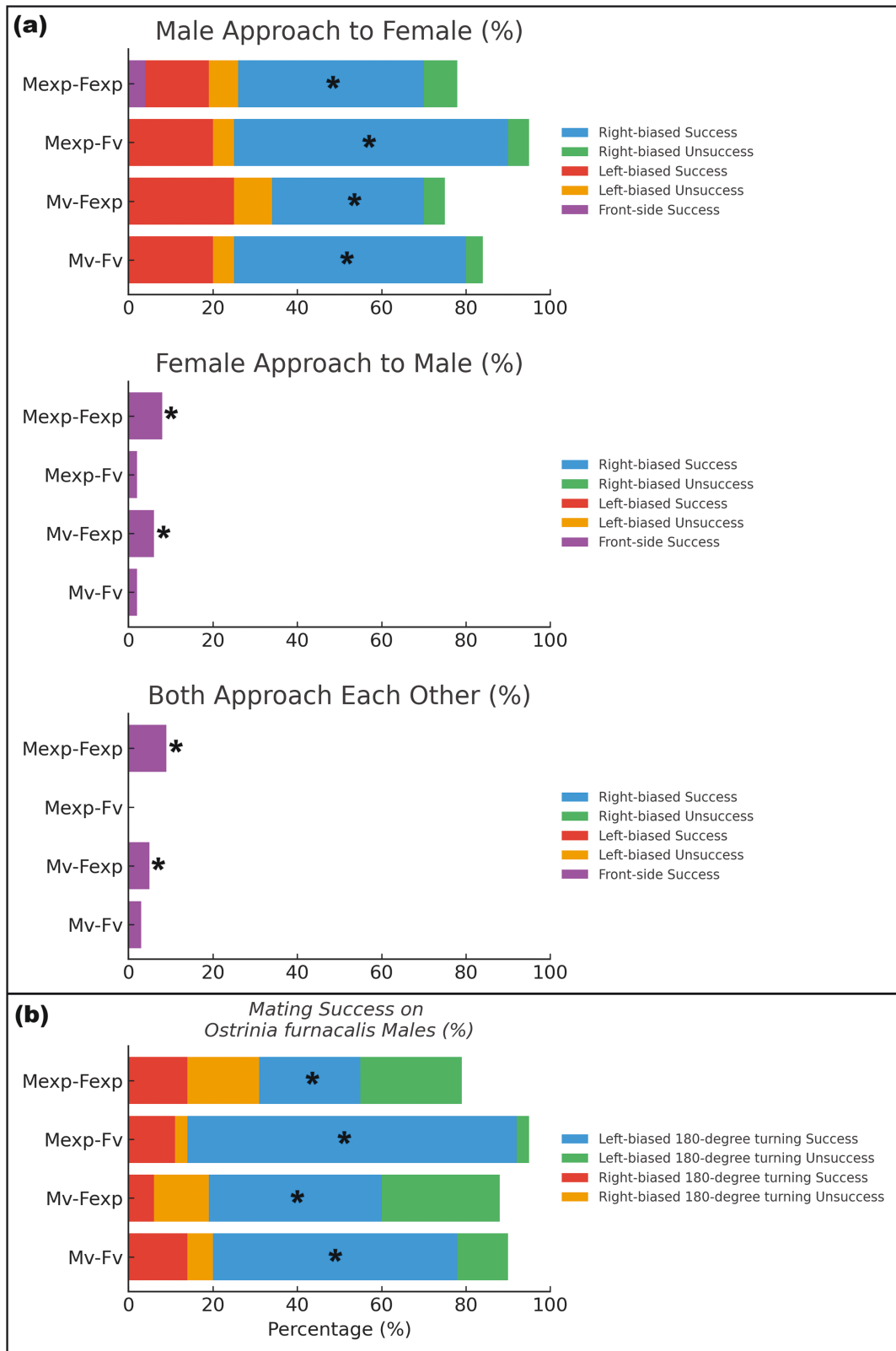


Fig. 3. Influence of adult (A) gender performing directional approaches to mate (B) males executing lateralized intromissive copulation with directional turnings and its impact on mating success of *Ostrinia furnacalis*. Asterisks indicate significant differences between (A) directional approaches and (B) directional turnings' impact on successful and unsuccessful mating (General Linear Model, Bonferroni correction, $P < 0.05$).

Table 1. Effect of gender and sexual experience on the total duration of the mating sequence in *Ostrinia furnacalis* adults (s)

Mating pair	Pre-copula (s)				Whole mating sequence (s)
	Mate recognition (s)	Male ultrasonic courtship song (s)	Male's antennal contact with the posterior of female (s)	Intromissive copulation (s)	
Mv-Fv	35.93 ± 12.21ab	60.16 ± 3.64ab	12.51 ± 8.82ab	4,173 ± 96.80ab	6.3 ± 45.8ab
Mv-Fexp	38.9 ± 10.7b	66.64 ± 7.32b	15.43 ± 17.26b	4,560 ± 102.32b	13.3 ± 25.8b
Mexp-Fv	75.20 ± 11.32ab	77.32 ± 19.43ab	24.3 ± 14.8ab	3,540 ± 88.23ab	9.3 ± 21.5ab
Mexp-Fexp	91.23 ± 8.32a	74.64 ± 11.34a	21.3 ± 29.7a	2,940 ± 92.54a	18.3 ± 19.3a
The mating pair: F, P	$F_{1,210} = 0.36, 0.7495$	$F_{1,210} = 0.17, 0.8115$	$F_{1,210} = 0.21, 0.6008$	$F_{1,166} = 0.99, 0.1897$	$F_{1,166} = 1.00, 0.2978$
Sexual experience of males: F, P	$F_{1,210} = 6.41, 0.0454 *$	$F_{1,210} = 4.89, 0.0500 *$	$F_{1,210} = 6.18, 0.0321 *$	$F_{1,166} = 1.40, 0.2411$	$F_{1,166} = 1.40, 0.2411$
Sexual experience of females: F, P	$F_{1,210} = 0.61, 0.6115$	$F_{1,210} = 0.21, 0.6455$	$F_{1,210} = 0.36, 0.5456$	$F_{1,166} = 4.80, 0.0312 *$	$F_{1,166} = 8.83, 0.0068 *$
Tested mating pair ($n = Mv-Fv, Mv-Fexp, Mexp-Fv, Mexp-Fexp$)	50 + 50 + 57 + 57 = 214				

Values are means followed by SD. Different letters within each column indicate significant differences in the time length of a specific mating phase among mating pair combinations; linear model, $P < 0.05$. Asterisks indicate significant effects of a specific variable on the time length in a mating phase; linear model, $P < 0.05$. Where no asterisks or letters exist, no significant differences were noted. Mv-Fv (male virgin and female virgin), Mv-Fexp (male virgin and female experienced), Mexp-Fv (male experienced and female virgin), and Mexp-Fexp (male experienced and female experienced).

the significant influence of sexual experience on the mating dynamics of both males and females, ongoing research should elucidate specific mechanisms underlying these behavioral patterns. This could include examining how variations in sensory processing and anatomical changes associated with experience further impact mating success, particularly in postcopulatory contexts.

Previous research on *O. furnacalis* revealed that males with right-biased approaches achieved higher mating success, particularly when performing left-biased 180-degree turns during intromissive copulation. Interestingly, no mounting from the back or front was observed; instead, males performed asymmetric turnings to achieve intromissive copulation, suggesting an alternative strategy to direct mounting (Abbas et al. 2024), a pattern similarly observed in some stored product beetles (Romano et al. 2016, Benelli et al. 2017a, Boukouvala et al. 2022). This study confirms that experienced males refine these lateralized movements over time, indicating that learning significantly enhances mating efficiency (Salva et al. 2012). However, context-dependent factors can modify these lateralized behaviors, leading to right-side copulation approaches (Romano et al. 2016, 2022, Frasnelli and Vallortigara 2018). Non-lateralized behaviors have also been observed in some insects, where males approaching from the front can achieve higher mating success than those turning 180° from either side, possibly due to more direct and efficient mate recognition (Calla-Quispe et al. 2023, Kavallieratos et al. 2023, Yin et al. 2023). A previous study demonstrated that lateralization in response to sensory and motor signals in *Prostephanus truncatus* is influenced by gender and sexual status (Romano et al. 2020). In *O. furnacalis*, both lateralized and non-lateralized behaviors improve copulation efficiency and adapt to environmental changes. This suggests that sensory inputs, learning, and memory play key roles in shaping male responses to mating signals, affecting their lateralized behavior (Carazo et al. 2004, Milonas et al. 2011).

The influence of lateralized mating behaviors on the duration of the mating sequence and its effect on copulation efficacy in our study showed that experienced males (Mexp) exhibited significantly longer durations of mate recognition and antennal tapping on the female's posterior compared to virgin males. The Mexp preference for approaching the female from the posterior not only increased the duration of contact but also contributed to a marked improvement in copulation efficacy. This suggests that a stronger non-lateralization confers a benefit in terms of improved motor control (Bell and Niven 2016, Frasnelli and Vallortigara 2018) or problem-solving (Güntürkün et al. 2020). The distinct copulation preferences of experienced males (Mexp) for specific left and right-side 180-degree turns, along with their reduced front-side approaches, indicate an adaptive strategy for increasing mating success. The preference for left-side turnings suggests a potential lateralized advantage in mating efficiency, as right-biased turnings were less effective. Furthermore, the reduced mating attempts by experienced males highlight a learned ability to recognize and correct ineffective mating strategies, emphasizing the role of sexual experience in refining mating behaviors and optimizing reproductive outcomes (Filice et al. 2021). Male laterality influenced the duration of key mating phases—mate recognition, copulation, and the overall mating sequence—in beetle pests such as *Cryptolestes ferrugineus*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum*, *Tribolium confusum*, *Trogoderma granarium*, and *Tenebrio molitor*. These behaviors are vital for reproductive success and population growth dynamics (Serrano et al. 2000, Romano et al. 2016, Boukouvala et al. 2019, 2020, 2021, 2022, Kavallieratos et al. 2023). Gender, sexual experience, and lateralization behavior significantly influenced mate recognition duration in *P. truncatus* (Romano et al. 2020). Furthermore,

Table 2. Effect of directional approaches on the duration of pre-copulatory behavioral displays in *Ostrinia furnacalis* adults (s)

Directional approaches	Mate recognition (s)			
	Mv-Fv	Mv-Fexp,	Mexp-Fv	Mexp-Fexp
Front-side (Fa)	--	--	--	24.0
Right-biased (Ra)	32.73 ± 2.56	36.97 ± 1.65	70.97 ± 5.47	89.87 ± 9.24a
Left-Biased (La)	29.32 ± 3.95	37.02 ± 3.52	67.34 ± 3.46	69.54 ± 12.78b
Directionality (<i>F, P</i>)	$F_{1,48} = 1.05,$ > 0.4341	$F_{1,48} = 0.53, 0.6629$	$F_{1,55} = 0.84, 0.5894$	$F_{2,54} = 6.09, 0.0500^*$
Tested Adults (n., Right + Left-Biased Adults)	0 + 10 + 40 = 50	0 + 12 + 38 = 50	0 + 12 + 45 = 57	0 + 16 + 41 = 57
	Male ultrasonic courtship song (s)			
Front-side (Fa)	--	--	--	35.0
Right-biased (Ra)	58.22 ± 1.45	67.65 ± 1.89	80.56 ± 1.02	87.34 ± 1.53
Left-Biased (La)	60.87 ± 1.56	58.97 ± 1.65	69.86 ± 0.94	69.98 ± 2.76
Directionality (<i>F, p</i>)	$F_{1,48} = 1.05, 0.2679$	$F_{1,48} = 1.67, 0.1643$	$F_{1,55} = 1.45, 0.2610$	$F_{2,54} = 7.11, 0.0491^*$
Tested Adults (n., Right + Left-Biased Adults)	0 + 10 + 40 = 50	0 + 12 + 38 = 50	0 + 12 + 45 = 57	0 + 16 + 41 = 57
	Male's antennal contact with the posterior of female (s)			
Front-side (Fa)	--	--	--	15.34
Right-biased (Ra)	12.76 ± 0.0.54	16.86 ± 0.1.32	23.74 ± 0.89	20.65 ± 1.58b
Left-Biased (La)	9.98 ± 0.0.87	14.45 ± 0.0.99	17.65 ± 1.12	18.87 ± 1.48a
Directionality (<i>F, P</i>)	$F_{1,48} = 1.45, 0.2610$	$F_{1,48} = 0.75, 0.7610$	$F_{1,55} = 0.95, 0.6612$	$F_{2,54} = 8.41, 0.0410^*$
Tested Adults (n., Front + Right + Left-Biased Approached Adults)	0 + 10 + 40 = 50	0 + 12 + 38 = 50	0 + 12 + 45 = 57	0 + 16 + 41 = 57

Values are means followed by standard deviations (SD). Different letters within each column indicate significant differences in the duration of directional approaches among precopulatory behaviors within a mating pair combination (linear model, $P < 0.05$). Asterisks indicate significant effects of directionality on pre-copulatory behaviors within a mating pair combination (linear model, $P < 0.05$). Where no asterisks are present, no significant differences were observed. Mv-Fv (male virgin and female virgin), Mv-Fexp (male virgin and female experienced), Mexp-Fv (male experienced and female virgin), and Mexp-Fexp (male experienced and female experienced).

Table 3. Effect of directional turnings on the duration of intromissive copulation in *Ostrinia furnacalis* adults (s)

Directional turnings	Mv-Fv	Mv-Fexp	Mexp-Fv	Mexp-Fexp
Front-Side (Fa)	--	--	--	--
Right-biased 180° Turning (Rbt)	4,173 ± 51.73	2,940 ± 72.87	3,173 ± 103.43b	2,940 ± 89.55
Left-biased 180° Turning (Lbt)	4,560 ± 67.97	2,940 ± 69.34	4,560 ± 122.52a	2,940 ± 90.87
Directionality (<i>F, P</i>)	$F_{1,47} = 1.00, 0.664$	$F_{1,47} = 0.08, 0.9872$	$F_{1,54} = 5.64, 0.0365^*$	$F_{1,54} = 0.12, 0.9130$
Tested Adults (n., Front + Right + Left-biased 180° Turning Adults)	0 + 10 + 40 = 50	0 + 12 + 38 = 50	0 + 12 + 45 = 57	0 + 16 + 41 = 57

Values are means followed by standard deviations (SD). Different letters within each column indicate significant differences in the duration of directional turnings within a mating pair combination (linear model, $P < 0.05$). Asterisks indicate significant effects of directional turning duration within a mating pair combination (linear model, $P < 0.05$). Where no asterisks are present, no significant differences were observed. Mv-Fv (male virgin and female virgin), Mv-Fexp (male virgin and female experienced), Mexp-Fv (male experienced and female virgin), and Mexp-Fexp (male experienced and female experienced).

the extended post-copulatory time observed in experienced mating pairs may enhance males' ability to fertilize females' eggs (Kamiya et al. 2015, Benelli et al. 2017b, Romano et al. 2018). These findings emphasize that sexual experience alters males' asymmetric mating responses, leading to greater efficiency in mating. As males gain experience, they adapt their behaviors, particularly favoring left-side approaches, which underscores the importance of learning in optimizing mating strategies and enhancing reproductive success.

Ultrasonic courtship song durations produced by males do not significantly influence copulation success in *O. furnacalis*. Instead, factors like the frequency of wing fanning and antennal tapping are more critical for reproductive success (Benelli et al. 2016). Wing fanning is a key courtship behavior in many parasitic wasps and is relevant to lateralized mating success and matching our findings (Benelli et al. 2014, 2020, Zeni et al. 2024). This behavior

may facilitate female recognition by increasing airflow around the antennae and enhancing pheromone detection (Loudon and Koehl 2000, Johnson et al. 2022). Furthermore, specific wing fanning frequencies may indicate male fitness to potential mates (Canale et al. 2013). Future research will explore the interactions between wing fanning, antennal tapping frequency, and mating success.

While our study did not specifically investigate lateralized leg kicking in *O. furnacalis*, we observed this behavior in both experienced and virgin females during the pre-copula phase. It appears to be an aggressive response to unwanted males, similar to behaviors described in other species. However, unlike mosquitoes, this kicking did not appear to displace partners effectively. The occurrence of leg kicking could be linked to males releasing sex pheromones during antennation, as seen in other Hymenoptera (Budriené and Budrys 2004, Reitmayer et al. 2021, Zeni et al. 2024). The lateralization

in kicking suggests a faster response rate, potentially linked to specific leg biases and differential nerve innervation (Tschida and Bhandawat 2015, Frasnelli 2017, Forrester et al. 2018, Knebel and Rigosi 2021, Zeni et al. 2024). Further research is needed to explore the lateralization in female leg kicking, and the morphological and neurophysiological factors underlying this behavior.

In conclusion, our research contributes to the understanding of lateralized courtship and mating behaviors exhibited by *O. furnacalis* during various mating phases. This study is the first to explore how gender and sexual experience influence both mating success and efficiency, demonstrating their crucial roles in non-lateralized and lateralized mate recognition. Specifically, experienced males, with a higher degree of lateralization in their mating approaches, achieved greater success than virgin males. In contrast, experienced females exhibited lower mating success rates and were less likely to remate, likely due to decreased receptivity or altered mating behaviors after copulation, highlighting the negative impact of sexual experience on their mating dynamics. Furthermore, the study reveals that the duration of mate recognition and the effectiveness of copulation are influenced by the behavioral asymmetries exhibited by both experienced males and females during their approach, underscoring the intricate dynamics of these mating interactions. Our findings also underscore the importance of sensory inputs, experience-based learning, and memory in shaping how adults process and respond to mating signals, influencing their lateralized behaviors. This research provides valuable insights into the ecological and behavioral dynamics of mate recognition and mating behaviors in *O. furnacalis*, particularly the influence of gender and sexual experience on lateralized mating behaviors. Understanding these dynamics lays the groundwork for exploring the neurological mechanisms behind these asymmetries, ultimately contributing to the development of more targeted and effective pest management strategies.

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Romano (Conceptualization [supporting], Validation [supporting], Writing—review & editing [supporting]), and Rizhao Chen (Conceptualization [Equal], (Funding acquisition [lead], Project administration [lead], Resources [Lead], Supervision [lead], Writing—review & editing [supporting]), Validation [Lead], Methodology [Equal])

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Conflicts of interest. The authors declare no conflict of interest.

Data Availability

All the data related to the research work are presented in the manuscript. Further details are available from the authors upon request.

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