The rapid release of sex pheromone reflects increasing willingness of female moths to mate

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Research Article

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Abstract

It is generally believed that female adults release sex pheromone to attract males in its calling behavior for mating. The female *Helicoverpa armigera* moth has a grade 1 ovary the day after emergence and develops into matured grade 3 on day 1. Mating peak is at Day 1 and 2, 1:00 of the circadian rhythm. The extrusion percentage and time of the ovipositor, typical calling signal, are lengthened as age increases, with the highest after Day 3 of age. The ovipositor extrusion elevates the release rate of sex pheromone by enlarging the releasing area, and after extrusion, the release rate increases with increasing ambient temperature. The peak of pheromone emission is on Day 3, 3:00 of the circadian rhythm. Mating time is earlier than pheromone biosynthesis, emission and release in female moths. After mating, the pheromone titer drops to a trace level before returning to its original level after 3 days. The activity time of male moths to sex pheromone is longer than that of females. We conclude that the accelerated release of sex pheromone increases a female moth's willingness to mate. If the female finds a conspecific male and mates successfully, the pheromone titer reduces to save energy.

Introduction

The cotton bollworm, *Helicoverpa armigera* (Lepidoptera, Noctuidae) is an important agricultural pest in China(Li and Rahmann 1997). Although the damage to the cotton crops has been reduced due to the planting of genetically modified cotton, the omnivorous pest has continued to break out and cause serious damage to other crops(Bilal et al. 2012; Huang and Li 2017; Yang et al. 2005). Its strong adaptability, high reproductive ability, and wide host range have made it a difficult pest to control(Ouyang et al. 2016). In recent years, it has occurred severely on corn, peanut, tobacco, pepper, fruit trees, and other crops. As *H. armigera* even quickly spreads across Northeastern China, the use of biological control is considered one of the priority technical options(Wyckhuys et al. 2013). Mass trapping and mating disruption through the application of sex pheromones are undoubtedly ideal tools(Burgio et al. 2020; Chamberlain et al. 2000; Kehat et al. 1998; Kehat and Dunkelblum 1993; Toyoshima et al. 2001). At the same time, the pheromone trapping for population forecasting is a necessary tool to reduce the application of chemical pesticides.

Understanding the mechanisms of pest reproduction plays an important role in the development of control and forecasting techniques and strategies to suppress and predict populations, and reproduction is a direct result of calling and mating behavior. It is generally believed that when insects are sexually mature, they release species-specific sex pheromones to attract conspecific individuals for mating by calling behavior. Sex pheromone of *H. armigera*, type I sex pheromone, was first identified as Z11-16: Ald (Gothilf et al. 1978; Piccardi et al. 1977), and later two trace components Z9-16: Ald (Nesbitt et al. 1979; Nesbitt et al. 1980) and 16: Ald(Wu et al. 1997) were also found to be synergists. The best ratio of Z11-16: Ald, Z9-16: Ald and 16:Ald in China is 97:3:4-6 (Wu et al. 1997), but 87:3:4 and 6% 16:OH in Isreal(Dunkelblum et al. 1980). Z9-14: Ald was identified as a synergistic compound as well (Zhang et al. 2012). Geographical differences have been reported in the ratio of Z11-16: Ald and Z9-16: Ald (Kumar and Shivakumara 2003; Tamhankar et al. 2003) and the difference in the inhibitory effect of Z11-16: Ac in

Australia and China, but not in Spain (Gao et al. 2020). However, despite the presence of Z11-16:OH in the solvent extract of the glands, the addition of Z11-16:OH has reduced the amount of moth catches in the field(Chen et al. 2018; Wu et al. 1997). Type I sex pheromone of lepidopteran moths is synthesized in sex pheromone gland at the end of the abdomen(Choi et al. 2005; Foster and Anderson 2020a; Jurenka et al. 1991), and then emitted outside the cuticle of the glands(Foster and Anderson 2020b) and released into the air through the rhythmic extrusion of the ovipositor(Liu and Haynes 1994; Schal and Carde 1985). Therefore, the extrusion of the ovipositor is the most important feature of calling behavior in female moths.

Z11-16:OH has been considered as a signal that the female moths are mature and can mate because they inhibit the attractiveness of sex pheromone of the *H. armigera* male moths. It is believed that the immature female moths of *H. armigera* release high doses of Z11-16:OH, while the content of Z11-16:OH in the mature female moths ready to mate will be greatly reduced (Chang et al. 2017). However, this statement was quickly raised in doubt. Z11-16:OH was actually not or very trace released outside of the body(Hughes and Carde 2020). Z11-16:OH should be an intermediate compound in the synthesis of Z11-16:Ald. It is reduced to sex pheromone Z11-16: Ald (Foster and Anderson 2019; Foster and Anderson 2020a). This also denies that Z11-16:OH is a signal compound for female moth ovaries to mature and mate (Hughes and Carde 2020). The question is, What is the relationship between female moth ovarian development and calling behavior? So far, the relationship between mating, calling and the production and release of sex pheromone is not clear in *H. armigera*. Currently, our understanding of the mediating mechanism of insect behavior overly relies on molecular study of olfactory genes, ignoring the chemical communications in intra- and inter- species and their association with behavior. Here, we analyzed the relationship between the temporal pattern and rhythm of ovarian development, mating, calling and the biosynthesis, emission and release of sex pheromone to further elucidate the association between mating and calling in *H. armigera*.

Materials And Methods

1.1 insects

H. armigera larvae are reared in the lab under the temperature 25±1°C and 80% ±5% humidity and fed an artificial diet. The pupae were collected and reared in an incubator at a temperature of 25±1℃ and a humidity of 60%±5%, and under a 11L:13D photoperiod (the light was off at 17:00 and on at 06:00AM). After eclosion, the male and female moths were separated and transferred into a 28cm x 28cm x 32cm folding insect cage and fed with 10% glucose water.

1.2 Observation of calling and mating behavior

The ovipositor extrusion and mating behavior of moths were observed every 20 minutes under a red light, and the start and end time of the ovipositor extrusion or mating were recorded. The percentage and time of ovipositors extruded was obtained from the observation of 6 females by 6 treatments. In mating bioassay, the newly emerging moths were paired with 10 females and 10 males in an insect cage.

1.3 Dissection of ovarian system

The abdomen of female moth was cut and then transferred into a petri dish filled with 50% alcohol, the cuticle was gently teared off with micro tweezers to get the ovaries exposed after the fat body and other tissues were removed, until its eggs were clearly observed. The ovarian grade was determined by the standard criteria(Feng et al. 2017, Zhang et al. 2013).

1.4 Extraction and quantification

Headspace extraction: The female abdomen was gently squeezed with the fingers to make the ovipositor extruded. The moth was moved towards one end of a glass tube with a diameter of 5mm and a length of 5cm, and the activated SPME fiber was inserted into the other end of the glass tube. After 5 minutes of adsorption, it is injected into a GC/MS for analysis.

SPME adsorption: SPME fiber (polydimethylsiloxane /divinyl benzene, PDMS/DVB, film thickness=100 μm, Supelco Inc., Bellefonte, PA) was used to absorb the pheromone emitted from the gland by rubbing back and forth on the cuticle outside the pheromone glands(Hughes and Carde 2020).

Solvent extraction: The female abdomen was gently squeezed to expose the sex pheromone glands, cut by an ophthalmic scissor, and then transferred into the 1 ml glass bottle. 50 μl of re-distilled analytical grade n-hexane was added into the bottle and the pheromone gland was removed after soaking in the solution for 1 h at room temperature. The extract was transferred to a glass lined tube with pointed closed bottom, and then concentrated to about 2μL with a minor nitrogen stream, and then was injected into the GC-MS.

Gland fluid extraction: The glass needle, made in PC100 (Japan/NARISHIGE) by drawing the glass capillary to a tip of 0.5 μm, was used to puncture the sex pheromone gland and suction the fluid in, and then transferred to the bottle. 50 μl of n-hexane was added. After immersing the solution for 1 h at room temperature, the supernatant was piped out and transferred to a glass lined tube with pointed closed bottom, and then concentrated to about 2 μl under a minor nitrogen stream, and was injected into the GC/MS.

The GC/MS instrument is an Agilent 8860GC-5977BMS equipped with a HP-5MS capillary column (30 m × 250 μm×0.25 μm). The program settings are as follows: 60°C for 1 minute, then at 10°C per minute to 180°C, then to 250°C at 3°C per minute and hold at 250°C for 10 minutes. The inlet temperature is 275°C, the ion source temperature is 230°C with an energy pressure of 70 e V, and the carrier gas is helium. The pheromone titer was quantified by standard sample.

1.5 Circadian rhythm of male moth trapping

A new designed automatic pest monitoring tool (Saipuxing SPT-F-02, NewCon Inc., Ningbo, China) daily and hourly monitored moth catches by pheromone trapping in the field. They were set up in the cotton-growing areas of five locations: Yanjin County in Henan (114°9'E,35°10' N)，Jinghai County in Tianjin

(117°7'E,38°46' N)Kuqa County in Xinjiang Uygur Autonomous Region ((82°53'E,41°30'N)Qitai County in Xinjiang Uygur Autonomous Region (89°31'E,43°51'N)

1.6 Data analysis

SPSS 17.0 software was used for data analysis. The means of multiple groups were compared using one-way analysis of variance, and the LSD method after ln (x + 1) transformation was used for significance analysis. The mean of the two groups was compared by Student's t-test. The effect of ovipositor extrusion was analyzed using Paired comparison test after ln (x + 1) transformation.

Results

2.1 The relationship between calling and mating

The ovaries of 0-day-old female moths was immature and developed at grade 1 when they emerged, but quickly developed into grade 2 on the same day. The ovaries of female moths are fully developed to grade 3 at 1 day of age (Fig.1).

The age of moths had significant effects on mating rate (F=11.08,*df*=29, *P*<0.001). The mating rate of 0-day-old male and female moth was only 2.5%, and the 1day old mating rate was the highest, 43.2±10.1%. The mating rate of 2 day old moths was 38.2±7.7%, and that of 3 day old was 13.9±4.5%. The mating rate of 1 and 2 day old moths was significantly higher than that of other ages, accounting for 81.4% of the total (Fig.2A).

When the female moth releases sex pheromone during calling, the ovipositor at the end of the abdomen will extrude outward in the dark period (Fig. 3a). The percentage and time of the ovipositor extrusion are related to the age of the female moths. The average extrusion time of day 1 moths was 1.8 ± 0.2 h, The average extrusion time to day 4 or 5 moths was the highest, 5.2 ± 0.2 or 4.9 ± 0.4 h (Fig. 2B). The 1day old female moth had a low extrusion rate of ovipositor and extruded late, starting after 0:00 and ending before 5:00, and the time lasted short. As age increased, the extrusion time was gradually earlier and longer, and the percentage increased. The highest number was observed at 3:00 in 2 day old female moths. 3 day old female moths began to extrude the ovipositor at 19:00, and the percentage of extruded ovipositor gradually increased, reaching the highest at 2:00. The ovipositor was extruded 100% after 23:00 for the 4-day-old and 5-day-old moths(Fig.2D).

The mating of male and female moths of *H. armigera* has a remarkable circadian rhythm (F=22.094,*df*=71,*P*<0.001). The mating percentages at 23:00 and 01:00 were 26.9%±4.2% and 31.0% ±2.1%, was significantly higher than other time periods. The mating percentages at 00:00 and 02:00 were 15.4±3.4% and 17.1%±2.1%, respectively, and the total mating rate between 23:00 and 01:00 accounted for 90.4% (Fig. 2C). Here, it indicated that the age of ovipositor extension was later than that of mating age, and in the circadian rhythm, the time of ovipositor extension was also later than the mating time. The percentage and time of ovipositor extrusion reached the maximum at 4 or 5 days of age.

When the ovipositor was not extruded, Z11-16: Ald was not detected or very minor in the headspace extraction by SPME(Fig.3d). After the ovipositor was extruded, the release of the major component Z11-16: Ald increased from 0.0009 ± 0.0005 ng to 0.0568 ± 0.013 ng at 25 °C, an elevation of 63 times compared to no extrusion (F = 26.2, *df* = 17, *P* = 0.006). When the temperature increased from 15 °C to 35 °C, the release amount was greatly elevated from 0.0138 ± 0.012 to 0.59 ± 0.263ng, which was 42.7 times higher than that at 15 °C (F = 6.965, *df* = 45, *P* = 0.002) (Fig. 3e).

2.2 Age and circadian rhythm in the titer of sex pheromone

Z11-16:OH was not detected in the headspace extraction and in the outer cuticle surface of pheromone glands by SPME adsorption, but only in the fluid of pheromone glands. Z11-16:Ald, Z9-16:Ald and 16:Ald were not detected in the fluid of pheromone glands. All components were detected in the solvent extract of pheromone glands(Suppl Fig.1). Female *H. armigera* moths at 0-day-old do not have Z11-16:Ald, and the release gradually increases after 1 day of age. At 3 days old, the maximum is 0.12±0.06 ng before the release amount starts to gradually decrease. The titer of Z11-16:Ald in the cuticle surface gradually increased with the increase of day age, reaching a maximum of 5.36±3.14 ng at 3 days of age, which was significantly higher than other days of age. Z11-16:OH in the solvent extracts of pheromone glands has the highest titer at the age of 3 days, which is 5.96±2.60 ng. The minimum titer at 0 days of age was 0.03 ± 0.02 ng。There was significant difference in the titer of Z11-16:OH between ages（F=4.168, *df*=23, *P*=0.011）. Z11-16: Ald titer is the largest when the female moth is 2 days old, 8.06±4.99 ng, but no significant difference in solvent extracts of different ages was analyzed(F=2.22, *df*=23, *P*=0.097). Z11-16:OH exist in the extracts of gland fluid of different female moths, there was significant difference between moth ages (F=3.219, *df*=23, *P*=0.03), the minimum titer at 0 days of age was 0.02±0.01 ng (Fig.4A).

In the headspace extract, there was no significant difference in the titer of Z11-16: Ald from 19:00 to 07:00 (F=1.60, *df*=33，*P*=0.185), and the titer gradually increased into scotophase. The highest peak was reached at 03:00, and the titer was 0.20±0.10 ng before gradually decreasing. When extracting the pheromone in the cuticle surface by the SPME adsorption, the highest peak was reached at 03:00, and the titer was 4.55±2.47 ng. However, in the solvent extraction of pheromone gland, the highest peak of Z11-16: Ald titer was 23:00, and the highest peak of Z11-16: OH was 01:00. In the inner fluid of gland, the highest titer of Z11-16:OH was at 03:00 (Fig.4B).

2.3 The effect of mating on the release of sex pheromone

After mating, the synthesis and release of Z11-16: Ald were suppressed significantly, and recovered slowly. At 24 hours after mating, the total pheromone titers extracted by solvent remained low, and Z11-16:Ald could not be detected by headspace and SPME adsorption. At 48 hours after mating, the total pheromone titer gradually recovered, but was relatively lower than just after mating. Z11-16:Ald could be detected in some of females by the headspace SPME (F=2.163, *df*=31, *P*=0.10). Z11-16:OH in the inner fluid of gland did not change significantly before and after mating(F=1.345, *df*=28, *P*=0.282). At 72 hours

after mating, the total pheromone titer significantly recovered, and reached the level just after mating (Fig. 5A).

However, for those female moths that mated earlier in the dark period, its sex pheromone was extracted by four methods at the normal release peak time (10 h into scotophase) in the same scotophase. The results showed that the titer of female moths decreased rapidly on the night after mating, and only solvent extraction had trace amounts of Z11-16: Ald and Z11 -16:OH(Fig. 5B). It was not detected in the sample with headspace SPME, and very trace amount in the sample with SPME adsorption. Z11-16:OH was not even detected in the gland fluid.

2.4 Circadian rhythm of male moth trapping in the field

The circadian rhythm of *H. armigera* male moth catches in 4 locations was recorded by pheromone trapping (Fig.6). In Xinxiang, Henan, on August 8-13, 2021, male moths were caught at the beginning of the dark period, reaching the peak of moth trapping 8 h into scotophase, and ending 10 h into scotophase. In Tianjin City on June 17-23, 2021, the circadian rhythm was the same. The moth trapping in Aksu, Xinjiang on July 9-16, 2021 started 2 h into scotophase, and the peak period was 7 h into scotophase. Male *H. armigera* moths in Changji, Xinjiang, on June 2-9, 2021 were trapped 1 h into scotophase, but there was no obvious peak catch of moths between 2 and 7 h into scotophase. Thus, the activity time of male moths to sex pheromone was 8 -10 h, which was more wider than that of female moths.

Discussion

The ovaries of *H. armigera* moths became sexually mature soon after emergence, and then they were ready for mating. The mating rate of day 1 and 2 after eclosion accounted for more than 80%. Meanwhile, only a very small amount of Z11-16: Ald in female moths was detected on the day of emergence. We found that sex pheromone released into the air varies greatly with the ovipositor extrusion in females. The percentage and time length of ovipositor extrusion were related to age of moths and the circadian rhythm. Even if the ovipositor of a 1-day-old female moth extruded, the time was relatively short. The highest percentage of 2-day-old moths extruding ovipositor was at 3:00, and the highest peak of major pheromone component Z11-16: Ald in the headspace was also at 3:00, which coincided in time. The temporal pattern of ovipositor extrusion was observed to be later than that of mating in terms of age and circadian rhythm. After the ovipositor is extruded, the exposed area of sex pheromone is dramatically enlarged, and its diffusion velocity is greatly increased. Moreover, our experiments also showed that its release rate after the ovipositor extrudes is positively correlated with the ambient temperature. Therefore, part of the reason for the extrusion is to release sex pheromone faster and in larger quantities, as reported in *Holomelina lamae* and *H. aurantiaca* (Schal and Carde 1985). From another perspective, it also indicates the strong desire of female calling for males while no males in the habitat. The older the female moth is, the higher the percentage of ovipositor extrusion, the earlier the time, the longer the extrusion time, which shows that the female moth has stronger calling behavior. It is inferred that the transfer of

sex pheromone to the cell membrane or epidermis after synthesis is physiologically controlled, but it is passively and naturally volatilized from the cuticle layer into the air outside once the ovipositor is extruded.

The biosynthesis of type I sex pheromone occurs in the sex pheromone gland, and the synthesized product is released into the cell membrane or epidermal layer (Foster and Anderson 2019; Levi-Zada and Byers 2021). In tobacco budworm *Chloridea virescens*, aldehydes are produced by similar alcohols through oxidases in the cell membrane or the cuticle layer of pheromone gland(Ding et al. 2014; Foster and Anderson 2020a; Foster et al. 2020; Hagstrom et al. 2013). It has been demonstrated here that, in *H. armigera*, solvent extract represents the total sex pheromone synthesized, the compounds emitted into the cuticle of pheromone gland was obtained in the SPME adsorption, while the sex pheromone actually released into air was in the headspace SPME. The results showed that only Z11-16: Ald, Z9-16 :Ald and 16:Ald are emitted to the cuticle surface, but Z11-16:OH does not. Sex pheromone was significantly biosynthesized and released at day 1. The highest pheromone titer was at day 2, but the highest amount emitted and released was on day 3. Thus, it can be concluded that the mating age is the same as the age of biosynthesis, but the time of maximum emission and release is one day later. Only a very trace amount of Z11-16:Ald is detected in the gland fluid. Z11-16: OH can only be detected in the internal fluid of gland and pheromone gland at all ages, which proved that Z11-16:OH is only an intermediate in the synthesis of sex pheromone in *H. armigera*, and has not been released into air(Hughes and Carde 2020). It is not related to ovarian development or mating signals of female moths, but obviously not as previously reported (Chang et al. 2017). Z11-16: OH has actually been confirmed to play a role in *C. virescens* sex pheromone (Groot et al. 2018) and maintaining species separation between *Chilo suppressalis* and *H. armigera* (Chen et al. 2018). Both sunrise and nocturnal insect behaviors have an innate circadian rhythm (Groot 2014), even for stored-grain pests(Zavodska et al. 2012). The gene expression levels of various enzymes that synthesize sex pheromone have circadian changes, such as turnip moth (Zavodska et al. 2009), beet armyworm(Cheng et al. 2010) and *C. suppressalis* (Guo et al. 2020), therefore, the circadian rhythm of sex pheromone titers is a common phenomenon (Bloch et al. 2013; Liu et al. 2013). The difference in the temporal pattern of calling behavior can also be one of the reproductive isolation strategies for insect species shared similar pheromone composition in the same habitat (Mazor and Dunkelblum 2005). From our results, the biosynthesis time of the sex pheromone of *H. armigera* was synchronized with the mating time, and the accelerated period was also close to the peak time of mating, that is, starting from 1 hour into scotophase, and the peak time for mating was 5-8 hours into scotophase. However, the peak time of the emission and release was at 9 hours into scotophase, which is significantly later than the mating time of male and female pairs. Meanwhile, the peak time for the release of sex pheromone occurs after the mating is over, which demonstrated the difference in temporal pattern between two. This may be because manually paired males and females do not need to be attracted by sex pheromone of conspecific female moths as in nature to mate.

The olfactory response of male moths to sex pheromone shows obvious circadian rhythms, not only in the expression levels of olfactory receptor genes (Zheng et al. 2014) and odor-binding proteins(Konstantopoulou et al. 2006), but in behavior as well (Kawazu et al. 2010). This behavioral

difference is not controlled at the level of the peripheral nervous system, but in the central nervous system (Rosen 2002), where octopamine may be involved in the process of signal recognition in the nervous pathway (Linn et al. 1996; Linn et al. 1992; Linn and Roelofs 1992). Compared with the time range of the pheromone release and mating in females, it is apparent that the active time range of the male moth's response to the sex pheromone is relatively wider than that of calling of conspecific females. It can start from the earliest period 1 hour into scotophase to 1 hour into photophase, and the peak period is 6 to 10 hours into scotophase, but it also showed that it is significantly affected by differences in generations, climates and geographic locations. Calling and mating circadian rhythms are related to age, temperature, relative humidity and photoperiod(Linn et al. 1996; Linn and Roelofs 1992; Wang et al. 2015; Xiang et al. 2018). Host plant not only affects the composition of sex pheromone, but also the circadian rhythm of the male olfactory response to sex pheromone(Groot et al. 2008; Schofl et al. 2009; Unbehend et al. 2013). The circadian rhythm of pheromone trapping in the field is a comprehensive effect of these factors. Therefore, the time difference between the female calling and male activity allows synthetic pheromone to effectively catch male moths in the field.

Generally, our understanding is that when insects are sexually mature, they first release sex pheromone into the natural environment to find the conspecific males(Sakurai et al. 2014). however, here we compare and analyze the temporal pattern of mating, calling, and release of sex pheromone. If the female moth release no or only a low amount of sex pheromone, it can easily find the male moth and mate successfully. There is no need for female moths to release a large amount of sex pheromone to find a mate. Only when the male cannot be found, the female will extrude the ovipositor to increase the release area, thereby to search for the males in a larger area. The older the virgin moth is, the longer the ovipositor extrudes, and the stronger the willingness to mate. Then, mating results in transient suppression of pheromone production in *H. virescens* (Ramaswamy et al. 1996) and *H. tea* (Raina et al. 1994) initiated by sperm or male accessory gland secretions. It has been demonstrated here that on the day after mating, the release of sex pheromone in *H. armigera* still changed by the circadian rhythm, but it was almost undetectable 24 hours after mating, and did not return to its original level until 72 hours later. The sex pheromone of females, whose mating was not fully completed, did not change 24 hours after mating, but no seminal vesicles were found after dissection of ovaries. It has indirectly proved the role of sperm in regulation of sex pheromone production.

In conclusion, the mating of the *H. armigera* moths depends on the maturation of the female ovaries, which is timely earlier than the release of sex pheromone. The onset of ovarian development, calling, pheromone production and mating of *H. armigera* are closely related to age and circadian rhythm. The emission of sex pheromone tends to temporarily stop after mating. Through the extension of the ovipositor, the release of sex pheromone is greatly elevated, and the ambient temperature increases the volatilization rate. Therefore, the release of sex pheromone does not indicate whether the female moth is ready to mate but reflects the strength of the female moth’s calling and mating willingness. This also verified that mass trapping and mating disruption must be applied at low density of pests and before emergence in the field.

Declarations

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Figures

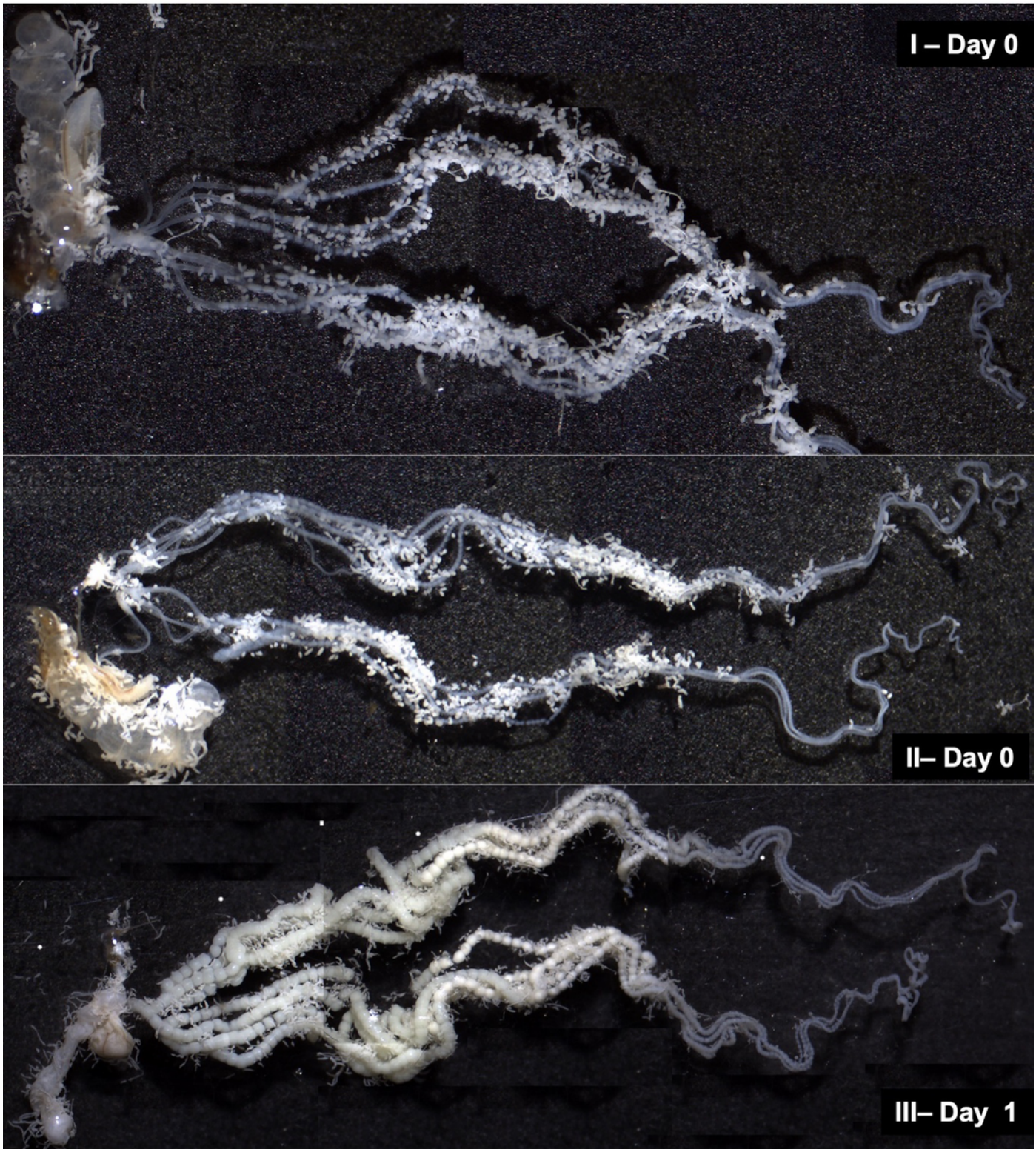

Figure 1

The development of ovary in the female moths aged 0-1 day. I, II - day0, III - day 1.

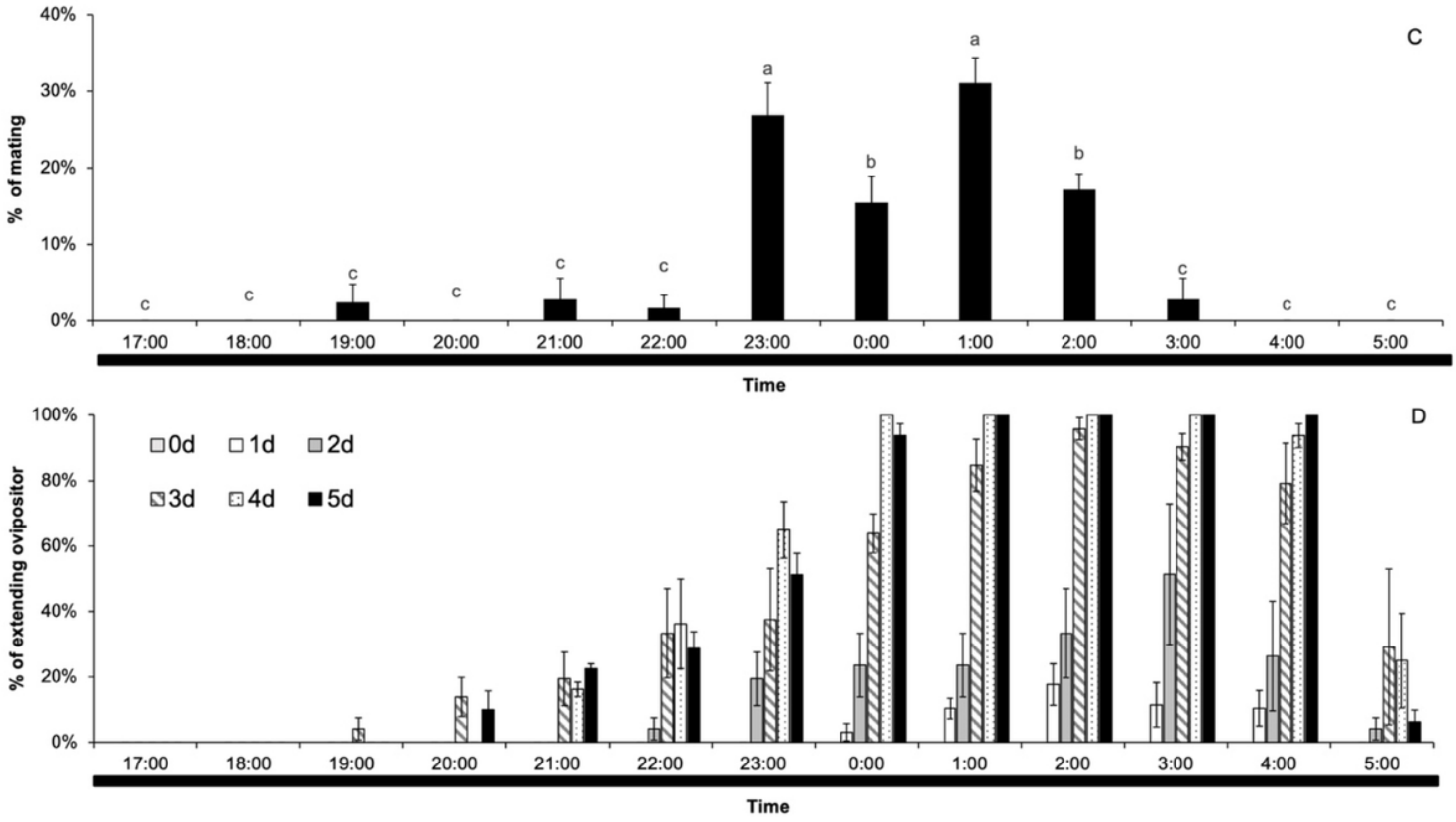

Figure 2

A Mating rate of male and female moths at different ages. B Mean time of ovipositor extrusion per night at different age of female moths. C Circadian rhythm of mating of male and female moths. Black bar represents the scotophase and White bar represents the photophase in the X-axis. D Percentage and circadian rhythm of the extrusion of the ovipositor of unmated female moths of different ages. Black bar represents the scotophase and White bar represents the photophase in the X-axis.

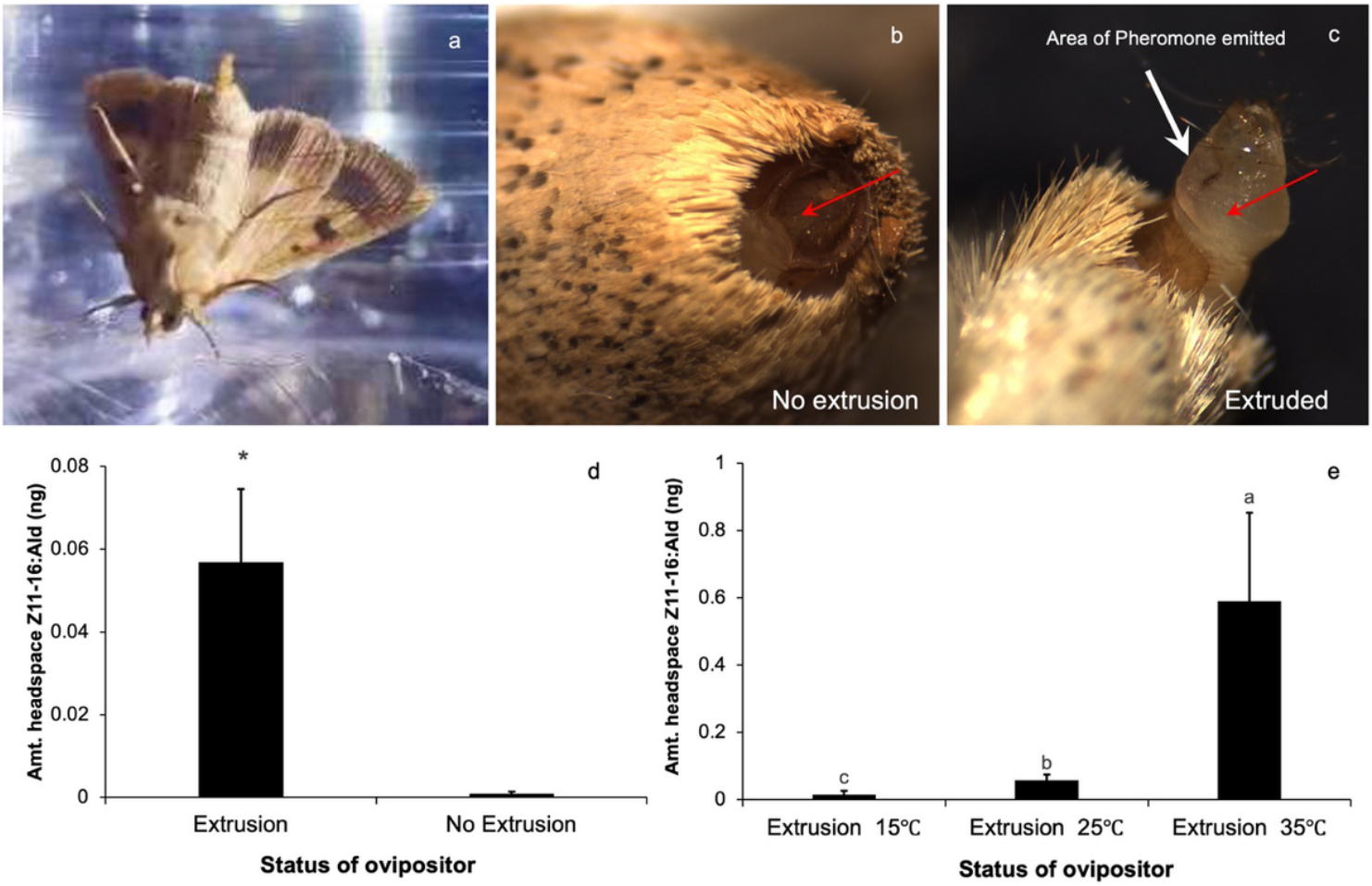

Figure 3

a calling female moth, b ovipositor with no extrusion, c ovipositor extruded, d Mean amount of Z11-16:Ald in the headspace volatile of ovipositors with no extrusion and extruded by SPME in 5 min, e Mean amount of Z11-16:Ald in the headspace volatile of ovipositors extruded by SPME in 5 min under different temperature environment.

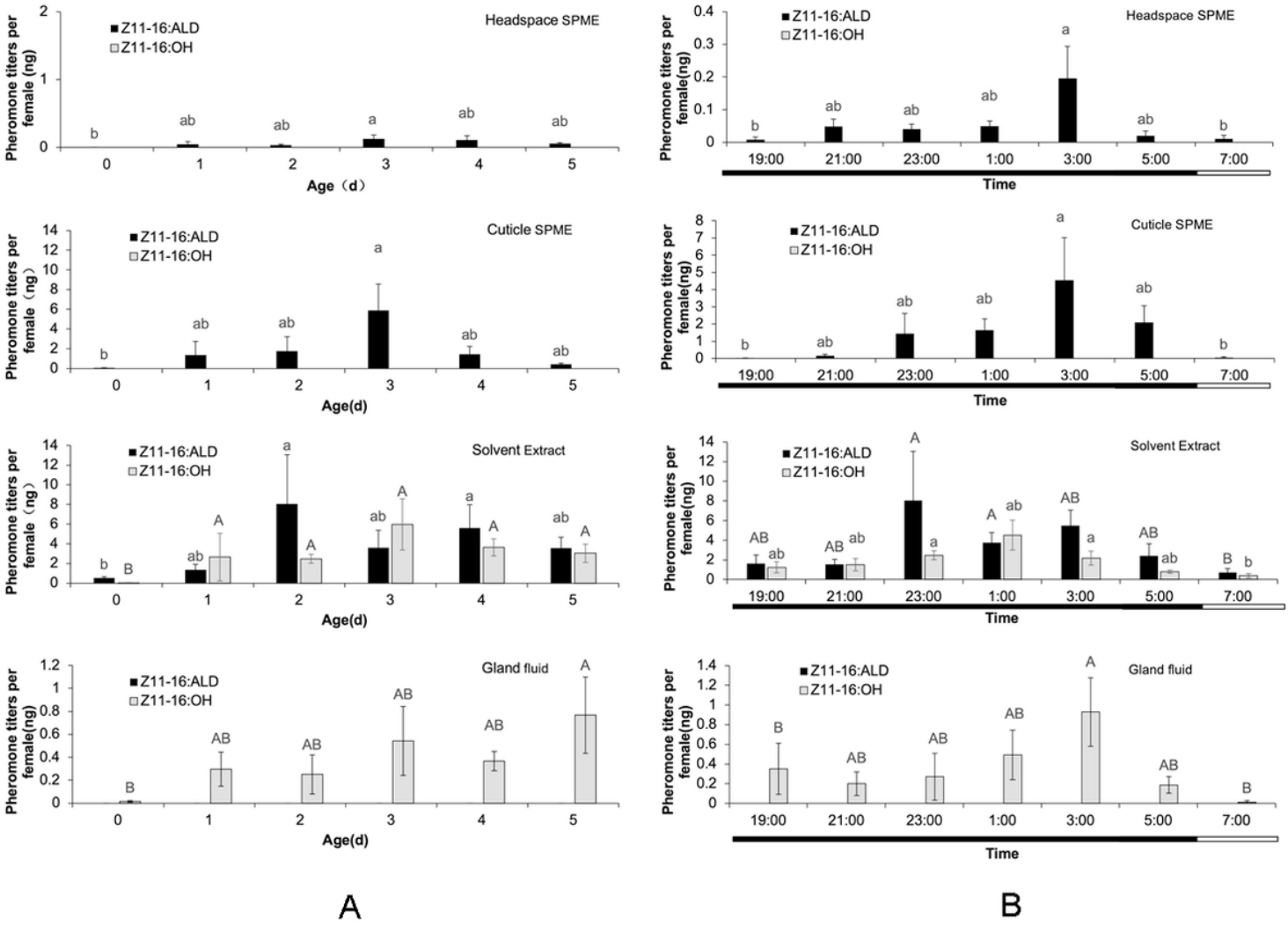

Figure 4

A, Pheromone titers of female moths from different parts at different ages. B Circadian rhythm of pheromone titers of female moths from different parts.

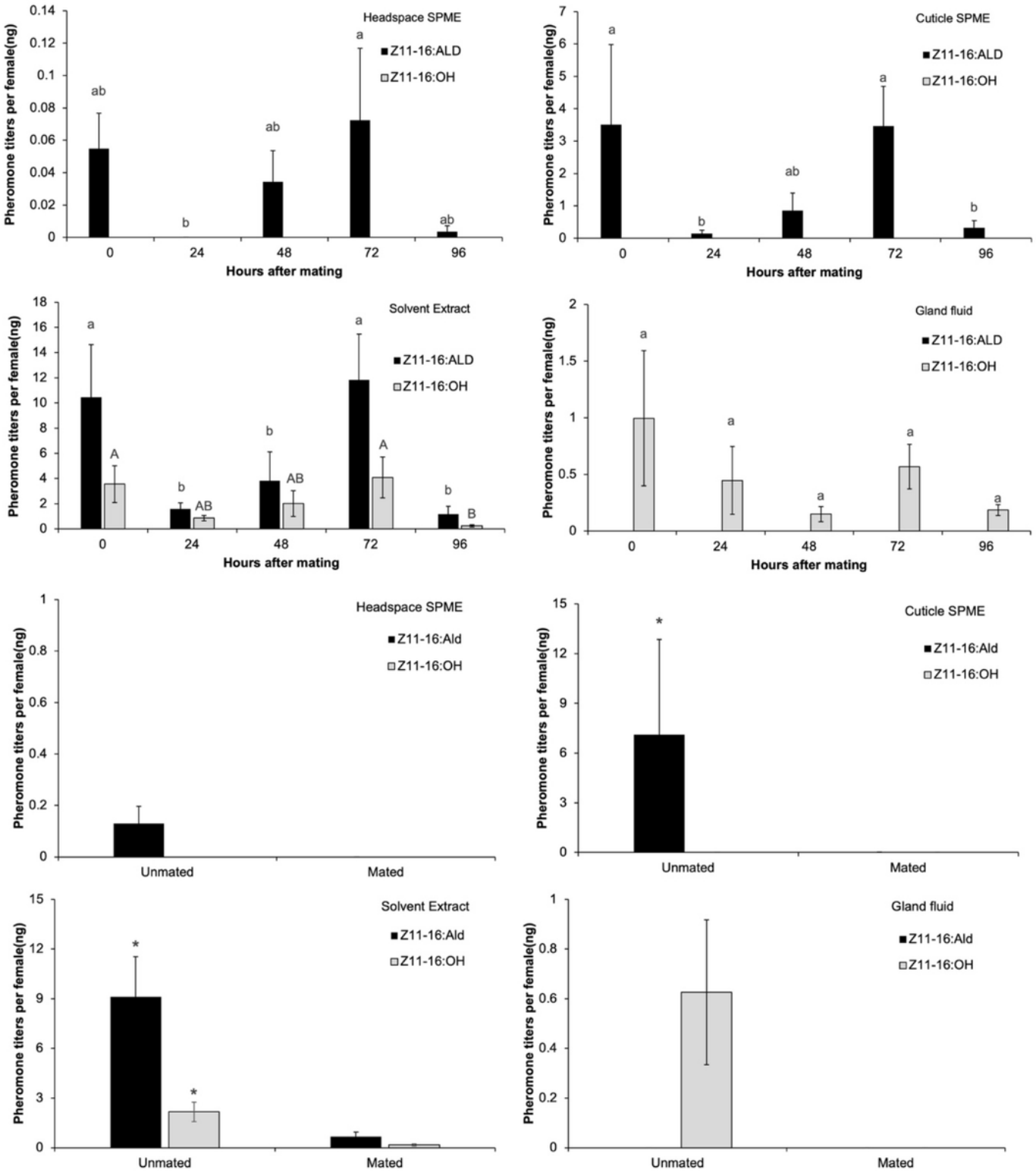

Figure 5

A Mean amount of Z11-16:Ald, Z11-16:OH in the headspace, cuticle surface, pheromone gland and gland fluid of female moths immediately after mating, 24, 48, 72 and 96 hours after mating. B Mean amount of Z11-16:Ald, Z11-16:OH in the headspace, cuticle surface, pheromone gland and gland fluid of female moths: a) un-mated, b) mated moths that mated earlier in the dark period, and sex pheromone was extracted 9h into scotophase in the same dark period.

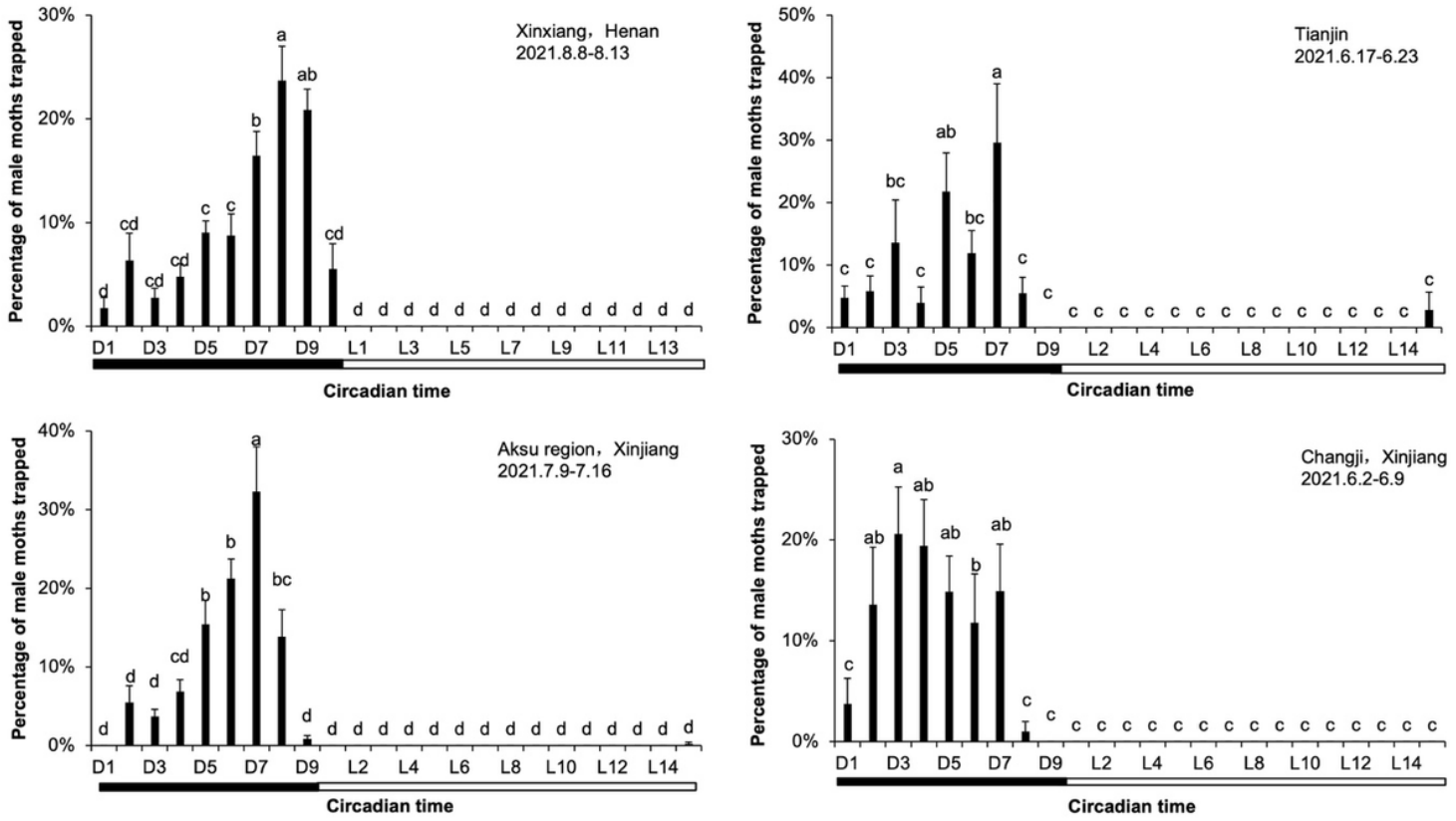

Figure 6

The daily number of moths caught and their circadian rhythms of hourly moth catches per trap in the field at the pheromone monitoring sites. D represents the scotophase and L represents the photophase in the X-axis.

Supplementary Files

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- SupplementalFig.jpeg