Chapter 5
Ultrasound-Induced Freezing Response in Moths

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Abstract Coevolutionary adaptation leads to modifications of sensory physiology and behavioral responses of predators and prey. From the ecological point of view, insect hearing and acoustic behavior are an attractive study area for researchers in entomology, animal behavior, and neuroethology. Recent technical advances in sound-recording equipment and molecular techniques have contributed to understanding the evolution of acoustic communication, including sexual dialogue, intraspecific competition, and the interspecific arms race between predators (e.g., bats) and prey (e.g., moths). Singing male moths exploit ultrasound-induced freezing responses of potential female mates or unwelcome rival males to enhance mating success. Freezing responses to ultrasound by moths are originally an antipredator reaction to echolocating insectivorous bats. The aim of Chap. 5 is to provide a sensory-behavioral explanation of the freezing response of insects during predator–prey interaction.

Keywords Bat-predator · Calling song · Courtship song · Freezing · Male–male competition · Moth-prey · Sensory exploitation

5.1 Introduction

Nocturnal insects often encounter predatory bats. Most insectivorous bats detect prey insects and obstacles by echolocation using intense ultrasonic cries. As a countermeasure, some insects (e.g., crickets, katydids, lacewings, moths, and tiger beetles) have evolved ultrasound-sensitive ears and defensive behavioral reactions (Miller and Surlykke 2001; Yack 2004). In general, flying insects turn away from

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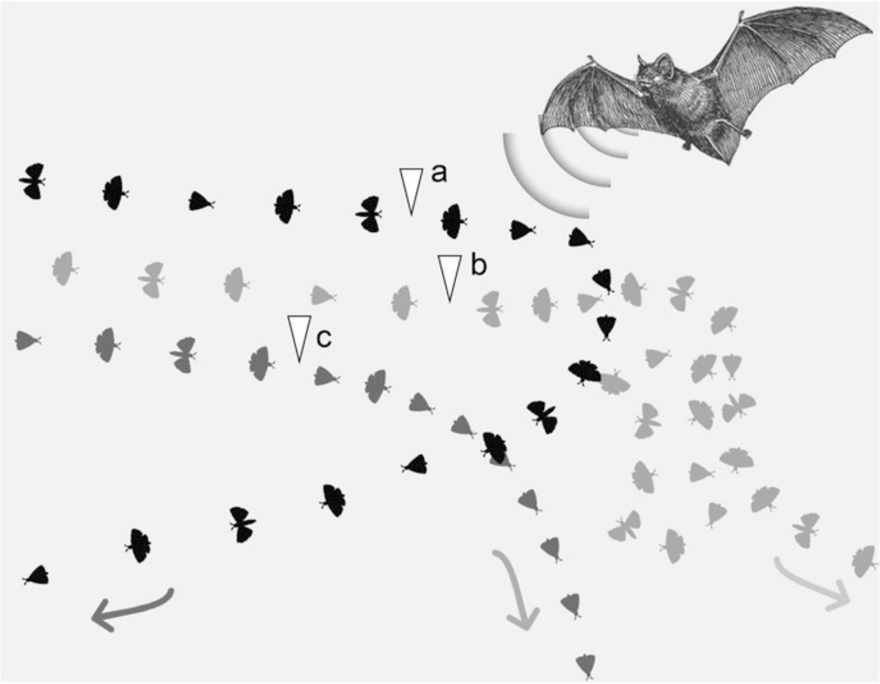

Fig. 5.1 Evasive maneuvers of eared flying insects in response to echolocation calls of a predatory bat. (**a**) turn-away flight, (**b**) zigzagging/spiral/looping flight (power dive), and (**c**) dive to the ground (passive dive). White arrowheads depict the onset of the ultrasonic pulses that insects can detect

the relatively infrequent and faint (but detectable) ultrasonic pulses of a distant echolocating bat (Fig. 5.1a). In contrast, frequent, loud ultrasonic pulses of a nearby bat in hunting mode induce last-ditch responses characterized by protean and erratic zigzagging, spiral, looping flight paths and diving to the ground by the targeted insect (Yager 2012; but see Nakano and Mason 2018) (Fig. 5.1b, c). The dive of prey insects is considered an adaptive defensive behavior to avoid bat predation because a bat chasing an insect in the air suddenly loses the echo reflected from the prey and thus has difficulty in orientating toward the diving insect. After diving and stopping wing movement, i.e., flight cessation, some insects such as moths do not show an immobility response and thus immediately resume flying or walking (Roeder 1962; Nakano et al. 2015a). Therefore, the passive dive could be considered an antibat freezing without tonic immobility. For “tonic immobility,” which is different from “freezing,” see Chaps. 1, 3, and 11; note that some erebid moths show tonic immobility (death feigning) by tactile and vibration stimuli (Chap. 3; Honma et al. 2015). Since the majority of aerially hawking insectivorous bats recognize a fluttering small object as prey, freezing is an effective defense against an approaching predator. Resting insects prior to the approach of a bat also utilize a motionless response (freezing) after detecting the bat’s echolocation calls and wait until the bat goes away.

Thus, roughly 85% of species of the night-active macro-lepidopterans develop a pair of tympanal organs which can respond to broadband ultrasonic wave including echolocation calls of predatory bats (Minet and Surlykke 2003). Moth ears show unique simple structure basically consisting of tympanic membrane, air-sac as a resonator behind the tympanic membrane, and only one to four auditory cells, A_1 to A_4-cell, and a non-auditory B-cell (for detail, see Yack 2004). Although the function of B-cell is still uncertain in sound detection, A-cells with similar frequency-tuning but different thresholds fire in response to mechanical vibration of the tympanic membrane stimulated by ultrasounds. Because a single hearing nerve holds all the A-cells and attaches to single point of the tympanic membrane, moth hearing is not characterized by the tonotopy which is seen in audition of other insects and animals, indicating that acoustic moths do not differentiate the tone of sounds, i.e., frequency (Nakano et al. 2015a). Eared moths, however, acquired both variable escape maneuvers triggered by echolocation sounds of bats and intraspecific acoustic communication in mating behavior and male–male competition. This chapter reviews the relationship between antibat freezing strategy and ecological aspects of acoustic signals of moths. In brief, because moths had developed ultrasound detectors after the divergence of the present lepidopteran superfamily, function and producing-mechanism of sound signals are often diverse in the same subfamily even. Some acoustic moths generate loud advertisement sounds serving as a calling song attracting potential mates and territory song repelling rivals while others generate loud or soft sounds serving as a courtship song attracting mates or freezing both mates and rivals.

5.2 Hidden Ultrasonic Communication

For lepidopteran insects, ultrasonic sexual communication was first reported in pyralid moths, the lesser wax moth, *Achroia grisella* (Pyraloidea, Pyralidae, Galleriinae), and the greater wax moth, *Galleria mellonella* (Pyraloidea, Pyralidae, Galleriinae), in the 1980s (Spangler et al. 1984; Spangler 1985). Sound production in tiger moths (Noctuoidea, Erebidae, Arctiinae; former Arctiidae) was reported as early as 1848 (Haldeman 1848), and a century later the sound was shown to be ultrasonic clicks (Blest et al. 1963). However, the authors described the function of the ultrasonic clicks as a defensive reaction against predacious bats, not as intraspecific communication signals. Up to the early twenty-first century, there are still only a few known examples of ultrasonic intraspecific communication. Thus, sexual acoustic communication has been thought to be quite rare in moths. However, this may be incorrect, based on a failure to notice mating sounds such as courtship songs in many moth species that use low-intensity and high-frequency sounds.

Loud acoustic signals, especially calling songs consisting of low-frequency sounds (<ca. 20 kHz), are advantageous to a distant receiver for sexual communication over long distances because of their low attenuation in the air. Hence, many insects, such as cicadas and crickets, use male calling songs to attract female

receivers that are far from the male signalers. However, conspicuous signals subject the signalers to eavesdropping by unintended receivers, such as predators, parasitoids, and conspecific rivals (e.g., satellite males) (Zuk and Kolluru 1998; Gerhardt and Huber 2002; Hedwig and Robert 2014; Legett et al. 2019). Predatory gleaning bats and eared parasitoid flies locate sound-producing insects using passive hearing (Belwood and Morris 1987; Zuk et al. 2006; Alem et al. 2011; Siemers et al. 2012). In contrast, the quiet courtship songs of male corn borer moths *Ostrinia* spp. (Crambidae) suggest that whispering "soft" ultrasonic courtship songs may be widespread among moths because they are less susceptible to eavesdropping (Nakano et al. 2008). A survey of 26 species of singing moths (Noctuidae, Erebidae, Pyralidae, Crambidae, and Geometridae) found that males of 11 species (42%) produced low-intensity (<80 dB peSPL at 10 cm) ultrasounds close to females with peak frequencies of 30–120 kHz (Nakano et al. 2009; Nakano and Nagamine 2019). Soft courtship songs were not found in all the species but were more common than previously thought. As shown in Fig. 5.2, the temporal and spectral features and sound pressure levels of the male courtship sounds are quite diverse, probably reflecting the different body parts involved in the two main types of sound production, stridulation by file-and-scraper (Fig. 5.2a, b) and clicking by tymbal buckling (Fig. 5.2c–e; presumably these males emit ultrasounds from tymbal organs). For the sound-producing organs of moths, see the previous review papers which described more details (e.g., Conner 1999; Greenfield 2014; Nakano et al. 2015a).

Among the noctuoid species producing courtship songs, metathoracic ventral tymbals are commonly found in male *Spodoptera* spp. (Nakano et al. 2010a; Nakano and Nagamine 2019) and metathoracic lateral tymbals are seen in tiger moths and lichen moths (Conner and Corcoran 2012; Nakano et al. 2013), but not in *Herminia* spp. and others (Nakano et al. 2009, 2015a). These findings suggest independent evolution of the sound-producing organs and ultrasonic signals. This corroborates the evolutionary scenario for the development of sexual communication sounds in moths: relatively recently, some moths have evolved male courtship songs after speciation of the ancestral species that had already developed functional hearing organs of which structures are shared in the same superfamily (Kawahara et al. 2019). Recent data combined with phylogenetic information indicate that whispering courtship song may be quite common in moths because it has the advantage of avoiding eavesdropping (Nakano and Nagamine 2019).

5.3 Calling Ultrasound

Ultrasound can also be a useful signal in moths to advertise the presence of the signaler. When they detect a nearby echolocating bat, tiger moths (Erebidae) and beggar moths (Geometridae) reply with ultrasonic clicks as antipredator defense (Conner and Corcoran 2012; Corcoran and Hristov 2014). Besides aposematic and jamming sounds above, some male moths generate loud sounds for intraspecific communication like common singing insects, cicadas, katydids, and crickets.

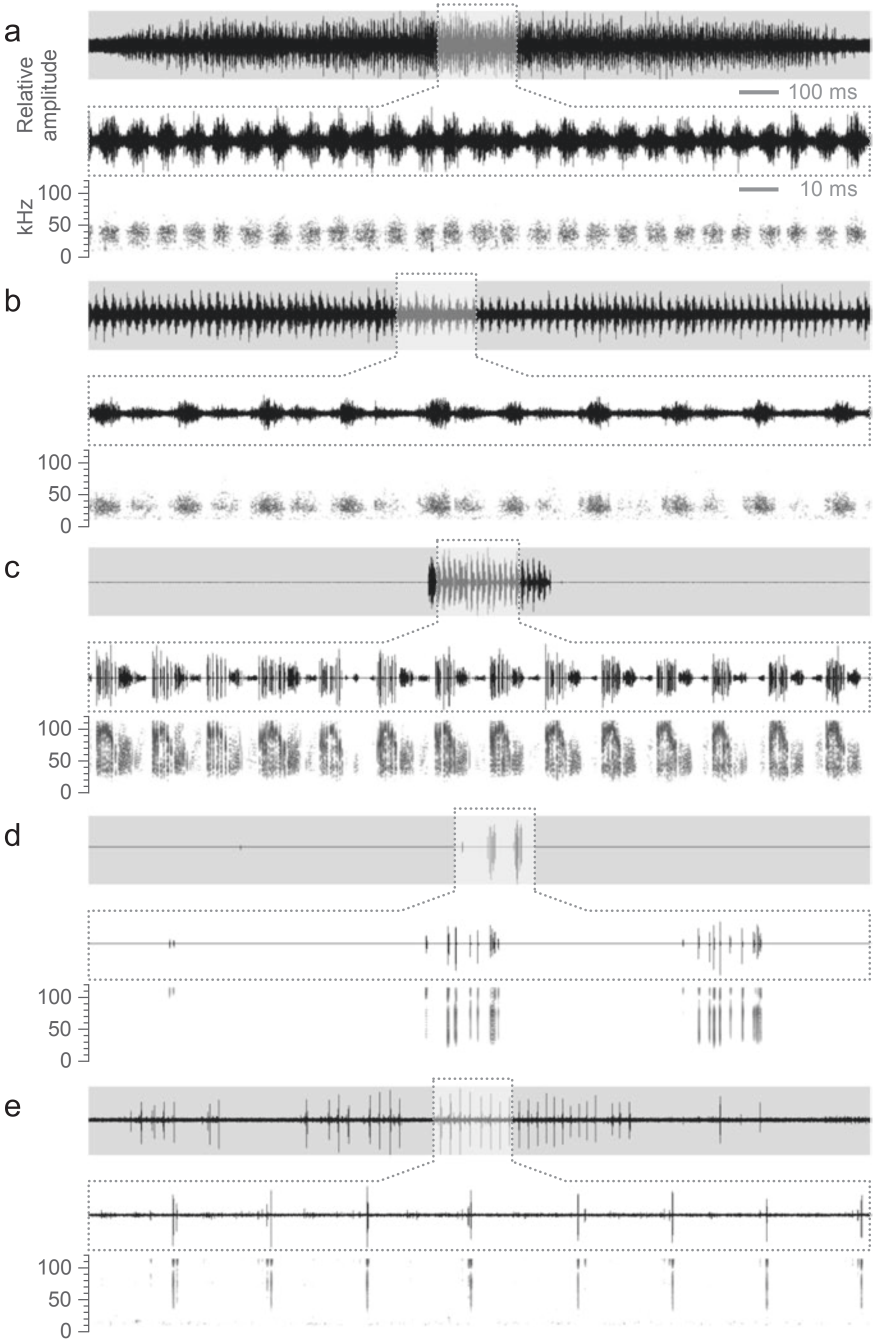

Fig. 5.2 Diversity of temporal and spectral characteristics of male sounds. Examples of male courtship songs in crambid moths: (**a**) *Ostrinia palustralis*, (**b**) *Ostrinia zealis*, (**c**) *Desmia funeralis*, (**d**) *Palpita nigropunctalis*, and (**e**) *Glyphodes pyloalis*. Oscillograms in 2 s (top) and 200 ms (middle; magnified area surrounded with box in top panel) and spectrogram (bottom) are shown. Adapted from Nakano and Nagamine (2019)

5.3.1 Mating System with Calling Songs

5.3.1.1 Noctuoidea

Males of diurnal Australian whistling moths, *Hecatesia* spp. (Noctuidae, Agaristinae), produce percussive sounds by striking knobs on the forewings ("alar castanets") together above the back (Bailey 1978). These sounds, which are partially ultrasounds emitted by males in flight, function as territorial signals against conspecific male rivals, and attract female mates (Alcock et al. 1989; Alcock and Bailey 1995). Males of *Rileyiana* (former *Phlogophora* and *Thecophora*) *fovea* (Noctuidae, Cuculliinae) rub a bubble (fovea) on the hindwing with the jagged hindleg (Surlykke and Gogala 1986). Perching males of *Amyna natalis* (Noctuidae, Eustrotiinae) also produce loud ultrasonic clicks from tymbal-like structures on the forewing (Heller and Achmann 1993). The functions of the male ultrasounds in the two nocturnal noctuids above, *R. fovea* and *A. natalis*, have not yet been determined, but they seem to function for attraction of female moths.

Males of the nocturnal Nolidae (Chloephorinae) moths *Bena bicolorana* and *Pseudoips prasinana* produce loud ultrasonic clicks with ventral tymbal organs located on the second abdominal sternite. The positive correlation between micro-tymbal structure and the number of clicks in these two species is similar to that in tiger moths: *B. bicolorana*, which has corrugated tymbals with striae on the medial part, produces a series of clicks for each buckling of the tymbal, whereas *P. prasinana*, which has smooth tymbals, produces a single click for each buckling of the tymbal (Skals and Surlykke 1999; Dowdy and Conner 2019). It is suggested that male ultrasonic clicks in the two nolid species are used as calling songs to attract female moths although no direct observations or experiments have been conducted.

In Erebidae, including the former Arctiidae (tiger moths and lichen moths), only a few species are known to use ultrasonic clicks produced from metathoracic tymbals for orientation before copulation (Conner and Corcoran 2012). Females of the polka-dot wasp moth, *Syntomeida epilais* (Erebidae, Arctiinae), release a sex pheromone at night to attract male moths, which is the typical mating system of moths. But remarkably, males start to sing a calling song after they begin to approach the females (Sanderford and Conner 1995). The female moth replies with ultrasonic clicks with a temporal structure different from that of the male's clicks, and the male reaches the female for mating. Mating sequence involving the "duet" is rather uncommon in moths.

5.3.1.2 Pyraloidea

In Pyraloidea (Pyralidae and Crambidae), there are several examples of loud ultrasound signals for calling females (Greenfield 2014). The lesser wax moth, *Achroia grisella* (Pyralidae, Galleriinae), is one of the most intensively studied moths for investigation of sexual selection and evolutionary processes in male calling song and female mate acceptance (e.g., Spangler et al. 1984; Greenfield and Weber 2000; Rodríguez and Greenfield 2004; Greenfield and Hohendorf 2009). Wing-fanning male moths emit a sex pheromone and ultrasonic clicks to attract female moths.

In Crambidae, males of *Symmoracma minoralis* (Pyraustinae) (Heller and Krahe 1994) and *Syntonarcha iriastis* (Odontiinae) (Gwynne and Edwards 1986) produce loud ultrasounds, which are most likely to work as calling songs. The sound-producing organs are located on the last abdominal segment and the genitalia, respectively, but the structure differs between the species: *Symmoracma* has a symmetrical tymbal and *Syntonarcha* has an asymmetrical file-scraper.

5.3.2 Evolution of Calling Songs

Noctuoidea moths possess homologous tympanal ears on both sides of the metathorax, which suggests that a common ancestor evolved ears once (Kawahara et al. 2019). In contrast, the diversity of sound-producing organs even within the same phylogenetic clade indicates that sound production has evolved independently several times (Conner 2014; Greenfield 2014). Sound-producing organs in moths that produce calling songs may be located in a variety of body parts, and the mechanisms may vary accordingly.

The temporal structure of calling songs of moths, including the duration of pulses (or series of clicks), interpulse interval, and duty cycle, is basically species-specific. The frequency components, however, commonly range from about 20 to 60 kHz and are a little higher in the smaller species such as pyralid moths; male *A. grisella* emits 70–130 kHz ultrasonic clicks. The dominant frequency of the calling sounds roughly matches the sensitive frequency range of the auditory cells, indicating that the frequency range of the calling song in moths is within the frequency range of the echolocation calls emitted by sympatric insectivorous bats (Schnitzler and Kalko 2001; Schnitzler et al. 2003). The incomplete tuning of the best frequency in hearing to the maximum energy in the calling song frequencies suggests that moths evolved acoustic communication by a sensory exploitation process after modification of ultrasound-sensitive hearing for bat detection (Nakano et al. 2013). The peak sound pressures of these advertisement sounds are usually greater than 90 dB peSPL at 10 cm. Based on hearing thresholds, attenuation in the air, and emitted sound pressure levels, the maximal communication distance is estimated at about 10–20 m. The loud sounds are therefore probably effective at medium distances, which are less than the effective distances of >200 m for volatile sex pheromones (Schlyter 1992) but are considerably greater than the distances involved in courtship.

5.4 Courtship Ultrasound

The majority of moths have not been thought to be acoustic insects with regard to mating behavior. Female moths release species-specific sex pheromones to attract conspecific male moths prior to copulation. After approaching the female releasing sex pheromone, the male attempts copulation. Thus, sex pheromones, which are effective in attracting a mate from a long distance, play an essential role in pair formation (Ishikawa 2020). This courtship sequence, which is described in textbooks, does not involve sounds; however, an increasing number of studies reports the role of ultrasonic songs in the courtship of male moths (Nakano et al. 2015a; Nakano and Nagamine 2019). Courtship ultrasounds produced by a male attracted to a female are found rather ubiquitously in moths, especially in Pyraloidea. The male moth sings a courtship song shortly before copulation attempts after orientation toward the female.

5.4.1 Loud Courtship Songs

Loud courtship songs are found in Pyraloidea. In the bee moth, *Aphomia sociella* (Pyralidae, Galleriinae), males attract conspecific females by sex pheromones ("male odor" or "male scent") and subsequently emit loud courtship songs in close proximity to the female to stimulate her to accept a mate (Kindl et al. 2011). The sound pressure level of the song reaches 96 dB SPL at 10 cm, which is about 1600-fold louder than the song of the Asian corn borer, *Ostrinia furnacalis* (Crambidae, Pyraustinae) (Nakano et al. 2008). Males of *A. sociella* have a mating territory and produce a rival song against intruding males, which is similar to the system in the Australian whistling moths, *Hecatesia* spp. (Noctuidae). In *Galleria mellonella*, which belongs to the same subfamily as *A. sociella,* males also emit ultrasonic rival songs against other males. The function of the rival songs in *Galleria* is still uncertain; females adopt a mate acceptance posture in response to rival songs as well as to courtship songs, indicating that females do not discriminate between the songs for rival males and female mates.

Male yellow peach moth, *Conogethes punctiferalis* (Crambidae, Spilomelinae), also emit loud courtship songs when they are close to a female. After approaching a female that is releasing sex pheromone, males hover around her and generate a series of brief pulses and then one long pulse (83 dB SPL at 10 cm) (Fig. 5.3a) (Nakano et al. 2012a, b). Only the long pulse, not the short pulses, causes the female to raise her wings upright and accept copulation (Fig. 5.3b). This specific wing-raising response is essential for copulation, as it triggers the male's landing and attempting genital coupling. Wing-raising is evoked by any long ultrasonic pulse, i.e., with duration >200 ms (Nakano et al. 2012a, 2014) (Fig. 5.3c). The series of short pulses with 28-ms pulse duration and 26-ms interpulse interval emitted before the long pulse does not directly affect the female's mate acceptance. However, the

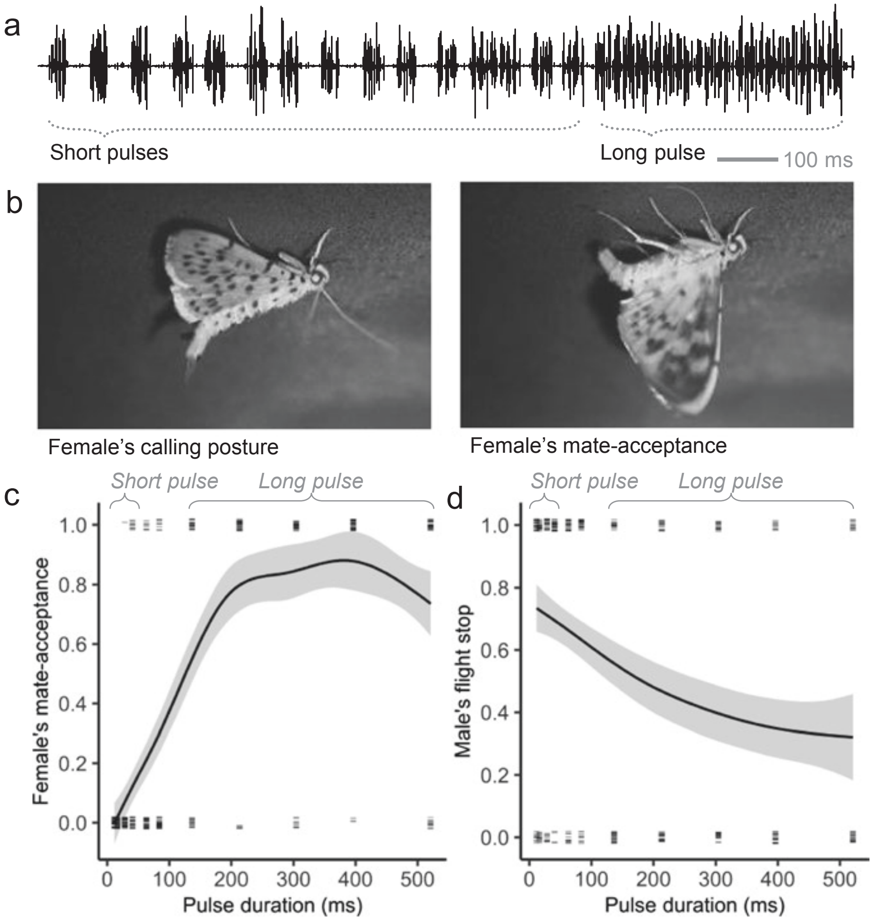

Fig. 5.3 Loud courtship song of the yellow peach moth, *Conogethes punctiferalis*. When close to a female, the male hovering around her generates a series of short pulses and one long pulse (**a**). Calling females adopt the mate acceptance posture (**b**) only in response to long pulses (**c**), whereas short pulses evoke cessation of the rival male's flight. Figure 5.3b is adapted with permission from Nakano et al. (2014)

temporal pattern is similar to that of echolocation calls in the approach phase of horseshoe bats, *Rhinolophus* spp., which frequently hunt moths. These calls elicit flight cessation in moths (Nakano et al. 2014, 2015b). Therefore, a possible function of the short pulses is to suppress the approach flight of rival males (Fig. 5.3d). Thus, male courtship songs of *C. punctiferalis* have a dual function to fend off rivals with the short pulses and make the female accept mating with the long pulse. The evidence that male moths produce loud ultrasounds before courtship to avoid interference from rival males in both *A. sociella* and *C. punctiferalis* suggests that the loudness of the male courtship song has primarily evolved for male–male competition. The high sound pressure level of the courtship song for communicating with the females might be a by-product of the use of the same sound-producing mechanism for rival songs and courtship songs.

5.4.2 *Soft Courtship Songs*

Males of the Asian corn borer, *Ostrinia furnacalis*, produce ultrasonic courtship songs of extremely low-intensity (46 dB SPL at 1 cm = 26 dB at 10 cm) in close proximity (≈1 cm) to a receptive female (Figs. 5.4 and 5.5a) (Nakano et al. 2006, 2008). The female's hearing threshold, 38 dB SPL, at their best frequency (46 kHz) allows females to detect the male song only when a male is singing within 3 cm of the female (Nakano et al. 2008). Males produce these sounds by stridulation; they rub specialized scales on their forewings against scales on the thorax (Fig. 5.4b) (Nakano et al. 2008). Relatively simple sound-producing scales on the body may have evolved more easily than other more elaborate cuticular sound-producing apparatuses such as tymbal organs, which require sophisticated modifications of the integument. The male songs suppress the escape behavior of the stationary female as she releases sex pheromones, thereby increasing the male's attempts and success in copulation with the motionless female (Nakano et al. 2010b).

Production of soft sounds has also been observed in other *Ostrinia* congeners (European corn borer *Ostrinia nubilalis*, adzuki bean borer *Ostrinia scapulalis*, etc.) (Fig. 5.2a, b). The sound pressure levels of male courtship songs are similar

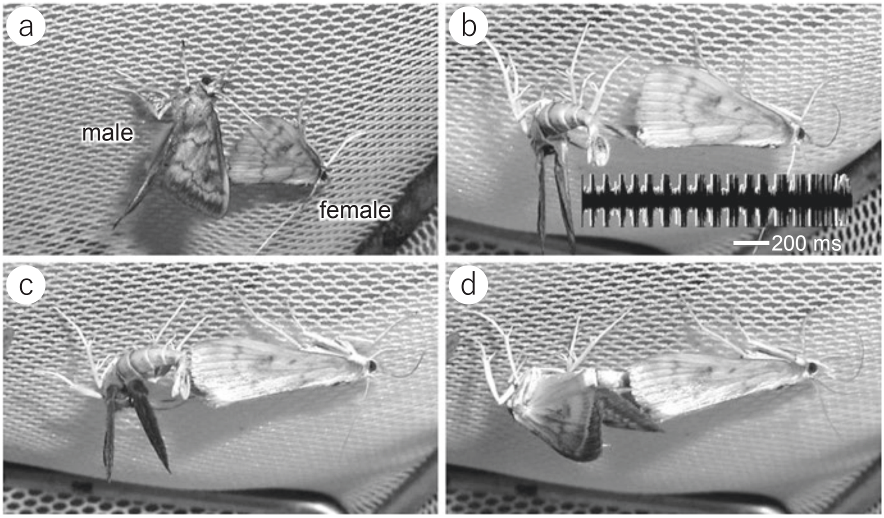

Fig. 5.4 Courtship sequence in the Asian corn borer, *Ostrinia furnacalis*. After approaching the female (**a**), the male erects both wings, rubs them against his thoracic scales (**b**) to produce soft courtship songs (inset oscillogram in **b**) shortly before attempting copulation (**c**), and copulates with the freezing female (**d**). Adapted with permission from Nakano et al. (2006)

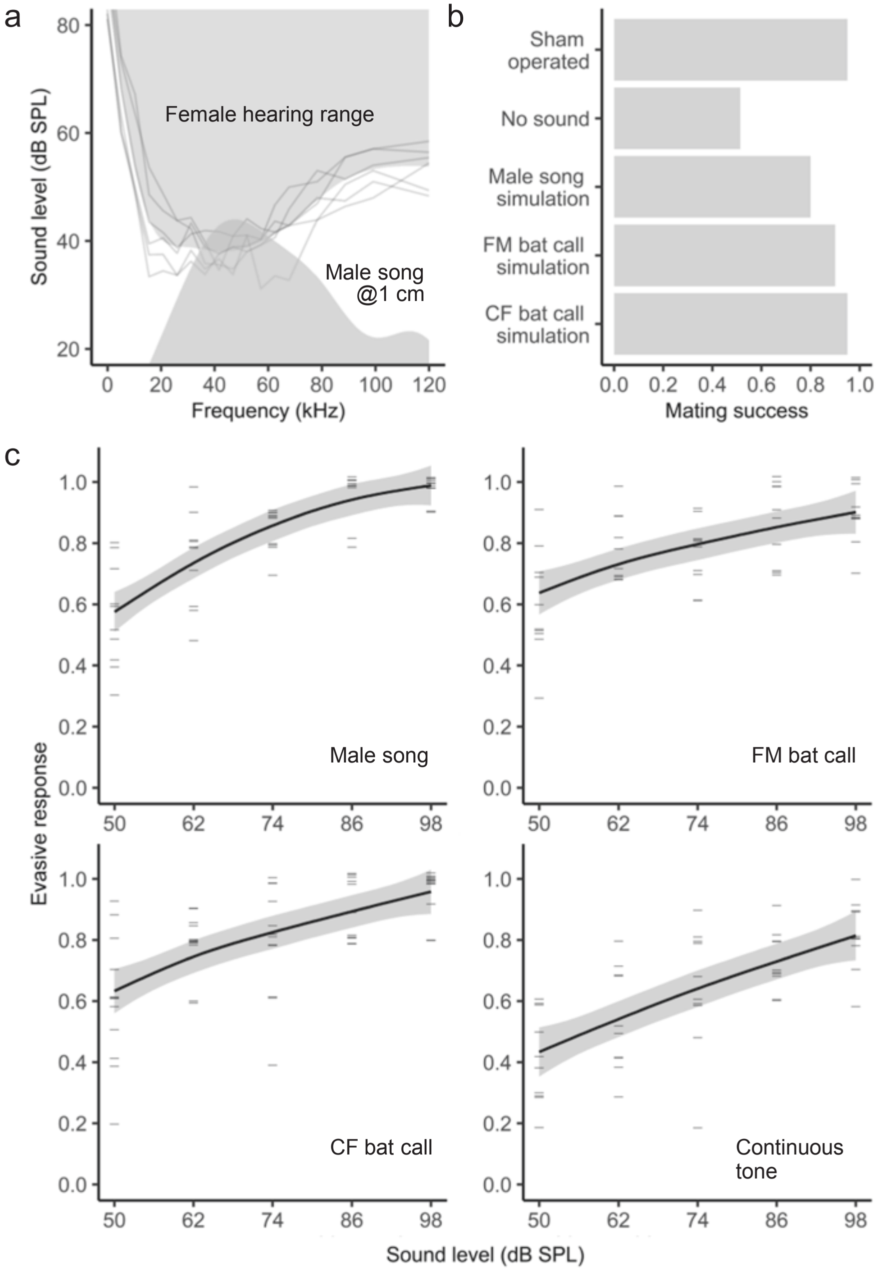

Fig. 5.5 Secondary use of freezing response in acoustic communication of *Ostrinia furnacalis*. (**a**) Spectrum of male ultrasound at a distance of 1 cm (at the female's location) corresponds to the most sensitive hearing range of the female moth. (**b**) Females accept muted males with simultaneous playback of male courtship song as well as simulated bat calls [frequency-modulated (FM) pulses and constant-frequency (CF) pulses]. (**c**) Rates of evasive responses to male song, two types of bat calls, and continuous tone in tethered flying moths. Adapted from Nakano et al. (2008, 2013, 2015a)

(23–26 dB SPL at 10 cm) and the hearing thresholds are also comparable (38–44 dB SPL at the best frequency) among *Ostrinia* spp. (Takanashi et al. 2010). Male courtship songs, thus, function only at very short distances in these species as well as *O. furnacalis* (Fig. 5.5a). However, the spectral features and temporal patterns differ, reflecting the phylogenetic relationships of *Ostrinia* species. The songs of male *O. nubilalis* and *O. scapulalis* consist of pairs of pulses (similar to Fig. 5.2a, b), whereas males of *O. furnacalis* emit pulse-group songs (Fig. 5.4b) with a higher frequency (peak frequency, 48 kHz; bandwidth 3 dB below the peak, 38–60 kHz) than in *O. nubilalis* (39 kHz; 31–45 kHz) and *O. scapulalis* (38 kHz; 29–45 kHz) (Nakano et al. 2006, 2008; Takanashi et al. 2010).

Although sounds are used for sexual communication in *Ostrinia* moths, the physiological threshold and hearing range have been conserved as a result of predatory pressures imposed by insectivorous bats (Takanashi et al. 2010). Thus, for these "soft-spoken" Crambidae, the most likely scenario is that evolution of ultrasonic hearing for bat detection came first, and later ancestral males of *Ostrinia* species acquired the ability to produce courtship songs that can be heard only by females in close proximity. The functions of male courtship songs are highlighted in the next section.

5.5 Secondary Use of Freezing Response

Moth acoustic communication is believed to have evolved through the receivers' preexisting ability to hear ultrasonic echolocation calls emitted by hunting bats ("sensory exploitation" or "receiver bias"; Endler and Basolo 1998; Ryan 1998; Greenfield 2014; Nakano et al. 2015a). Thus, prominent sound production for mating is restricted to eared moth species. The use of mating sounds via exploitation of a sensory bias, however, involves more than just detecting the sounds.

5.5.1 True Love Songs

Modification of opposing behavioral responses between aversion maneuvers against predatory bat calls and positive orientation toward and acceptance of a mate's signal is also essential for the evolution of sexual communication in general. For example, in the lesser wax moth, *A. grisella*, female moths show negative phonotaxis to echolocation calls of bats but are attracted to male ultrasonic calling songs (Rodríguez and Greenfield 2004; Greenfield and Weber 2000; Greenfield and Hohendorf 2009). In the lichen moth, *Manulea* (former *Eilema*) *japonica* (Erebidae, Arctiinae), and the yellow peach moth, *C. punctiferalis*, females discriminate between bat echolocation calls and male courtship songs on the basis of the temporal structure of sound signals and adopt the mate acceptance posture to male courtship songs of their own species (Nakano et al. 2013, 2014). Nevertheless, except for moths like the two species above, *M. japonica* and *C. punctiferalis*, the requirement of behavioral reversal (negative to bat calls and positive to male songs) might not be critical in the courtship context. Courtship song functions at extremely short range, which renders positive orientation unnecessary. Hence, addition of courtship song to the typical moth mating system has probably made courtship singing more common than loud calling song in moths. Adaptive elaboration of moth acoustic communication through sensory exploitation results in tuning of the characteristics of the sender's sounds to the detection abilities of the receiver, e.g., detectable sound intensity and high-frequency sounds of bat echolocation calls. Subsequently, both the sender's signal and the receiver's response have coevolved by way of sexual selection (Greenfield 2014). Thus, a prerequisite for establishing sensory exploitation in moths is to demonstrate that the receiver's preference for the mate's communication signal evolved after the development of evasive action in response to bat ultrasounds. This means that it is extremely hard to corroborate the sensory exploitation experimentally by using species that have evolved a receiver's preference for the sender's sound signal. For Arctiinae moths of which males generate courtship songs and both sexes generate antibat aposematic/jamming signals, female's preference for male courtship songs is likely to have evolved after evolution of the aposematic and jamming sounds against echolocating bats.

5.5.2 Deceptive Love Songs

Males of *Ostrinia furnacalis* and the common cutworm, *Spodoptera litura* (Noctuoidea, Noctuidae, Noctuinae), emit soft courtship songs with low sound pressure levels (see Sect. 5.4.2). The females do not distinguish male songs from bat echolocation calls. When not only playback of male courtship songs (46 dB SPL for *O. furnacali*s and 70 dB SPL for *S. litura* at the position of the female's ear), but also simulation of bat echolocation calls (74 dB SPL for *O. furnacali*s and 100 dB SPL for *S. litura*) was presented to the receptive female moth, aphonic males with ablated sound-producing organs were not rejected by females in the courtship context and could complete fertile copulation (Fig. 5.5b) (Nakano et al. 2010a, 2013). The behavioral responses of females to bat calls and conspecific male songs are similar. They "freeze" when they hear either sound, which is one of the maneuvers that aids resting moths to avoid capture by the insectivorous bat's biosonar. By secondarily exploiting the female's freezing response to ultrasounds, the singing male can persistently attempt genital coupling and thus increase his copulation success because the female is made motionless. Regardless of sex, females and males of *O. furnacalis* in flight also freeze, i.e., dive to the ground, or take evasive maneuvers in response to simulations of echolocation calls of the big brown bat, *Eptesicus fuscus* (Vespertilionoidea, Vespertilionidae, Vespertilioninae), and the greater horseshoe bat, *Rhinolophus ferrumequinum* (Rhinolophoidea, Rhinolophidae, Rhinolophinae), which are representative insectivorous bat species. In addition to bat call simulations, playbacks of male courtship song caused bat avoidance behavior in flying moths if the playbacks were presented at >70 dB SPL at the location of the moth (Fig. 5.5c). Because males of *O. furnacalis* and *S. litura* do not generate loud courtship songs, flying moths do not exhibit drastic reactions, such as protean and unpredictable flight responses, to their actual courtship songs. It is therefore inferred that the female interprets a male's soft courtship song as an echolocation call emitted by a distant bat and remains stationary in order not to be detected by the bat. The identical reactions of females to bat echolocation calls and male courtship songs support the hypothesis that eared moths evolved acoustic sexual communication based on a preexisting sensory bias.

5.5.3 Evolutionary Origin of Courtship Songs

After acquiring hearing sensitive to ultrasound and defense strategies as a countermeasure to echolocation in predatory bats emitting ultrasonic pulses, there were probably some males that incidentally generated weak noise, including sounds in the ultrasonic range, in courtship displays, such as wing-fanning for diffusing the male sex pheromone to the intended female or collecting the female sex pheromone (Obara 1979; Spangler 1988; Kindl et al. 2011). This assumption implies that females were able to detect the by-product ultrasounds, which caused a freezing response and thus led to increased copulation success. This process accelerated the use of bat-like male songs in courtship because of the initial lack of females' preference and/or recognition of an appropriate conspecific mate on the basis of male song characteristics, with a mainly species-specific temporal structure. In contrast to the deceptive courtship song, if the acoustic features of males' original mating sounds were extremely far from those of bat calls, females would not show antibat reactions to these sounds. This is why moths do not always take evasive action to all the detectable sounds (Nakano and Mason 2018). A species that had been able to distinguish male courtship sounds from bat echolocation calls and to increase mating success could evolve female mate preference and recognition and develop "true" love songs via coevolutionary sexual selection (Endler and Basolo 1998; Ryan 1998; Greenfield 2014).

There are an increasing number of case studies of moths in which the males produce ultrasounds during courtship (Conner 1999; Nakano et al. 2009; Greenfield 2014; Nakano and Nagamine 2019). The functions of most of these courtship ultrasounds are still unclear. Experiments are warranted using behavioral tests with moths in which the hearing organs or sound-producing organs have been ablated and using playbacks or simulations of courtship ultrasounds. Thus, it is unknown whether males' ultrasounds are used for sexual communication with females, for resource competition with rival males, or both. Even if male courtship song serves as a mating signal, the mode of action should be determined by comparing behavioral and auditory responses to bat echolocation calls and male courtship songs. The function of moth courtship songs is divided into two types on the basis of the receiver's reaction. The "deceptive" function is found in the crambid *O. furnacalis*

(Nakano et al. 2013) and the noctuid *S. litura* (Nakano et al. 2010a), and the “true” attractive function is found in the pyralid *G. mellonella* (Spangler 1985) and the erebid *M. japonica* (Nakano et al. 2013). Only in the case of “true” mating signals, receptive females discriminate between bat echolocation calls and male courtship songs and thus perform a freezing response to bat calls but not to male songs.

It is difficult to conduct persistent observations of courtship behavior of animals with uncertain times of day for mating activity. Nocturnal moths usually produce soft and brief courtship ultrasounds that are not audible to the human ear (Nakano and Nagamine 2019). Our sensory bias toward low-frequency (<20 kHz), loud, and long duration sounds has contributed to the failure to detect the actual number of insects communicating with soft courtship songs (Balenger 2015; Reichard and Anderson 2015). Future studies are anticipated to reveal the use of soft sounds for private communication in more moths as well as other animals. Subsequently, these mating sounds will be confirmed to be deceptive or true signals. These studies will be essential for deepening our understanding of the evolutionary scenario of moth acoustic communication. To demonstrate that females have acquired the current mate preference to male sounds from original negative responses to predator signals may contribute to our knowledge of the evolution of novel communication modalities.

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