# Moths produce extremely quiet ultrasonic courtship songs by rubbing specialized scales

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Insects have evolved a marked diversity of mechanisms to produce loud conspicuous sounds for efficient communication. However, the risk of eavesdropping by competitors and predators is high. Here, we describe a mechanism for producing extremely low-intensity ultrasonic songs (46 dB sound pressure level at 1 cm) adapted for private sexual communication in the Asian corn borer moth, Ostrinia furnacalis. During courtship, the male rubs specialized scales on the wing against those on the thorax to produce the songs, with the wing membrane underlying the scales possibly acting as a sound resonator. The male's song suppresses the escape behavior of the female, thereby increasing his mating success. Our discovery of extremely low-intensity ultrasonic communication may point to a whole undiscovered world of private communication, using "quiet" ultrasound.

acoustic communication | hearing | playback experiment | receiver bias | sound-producing organ

**Fig. 1.** Synchronicity of ultrasonic song production and wing strokes in *O. furnacalis*. Gray line represents the oscillogram of six sound pulses recorded with a microphone during courtship. Black line with dots shows a trace of distances between distal edges of the right and left forewings, calculated from a frame-by-frame analysis of high-speed video (Movie S2) at 2-ms intervals (31 frames in 60 ms), which indicates up and down strokes of the wings (white and black arrows, respectively).

Insects have evolved methods of communication, using conspicuous sounds. However, the risk of eavesdropping by competitors, predators, and parasitoids is high (1, 2). To counteract this risk, various signalers (e.g., crickets, katydids, and moths) have changed the temporal and spectral characteristics of their signals and the timing of signaling (2–5). In addition, several katydids are known to exploit sensory signals outside of the eavesdropper's sensory channel, e.g., vibrational signals (2, 3). Another conceivable strategy to alleviate the risk of eavesdropping is to minimize the signal intensity (6) and whisper at the counterpart's ear. However, few insects are known to adopt this strategy.

The great majority of moths have ultrasound-sensitive tympanal ears, which probably have evolved for detecting ultrasonic echolocation calls of insectivorous bats, thereby avoiding predation (1, 7, 8). Intraspecific acoustic communication using ultrasonic signals, which might have evolved through the exploitation of receiver bias (1, 7), was reported in a small fraction of these tympanate moths (reviewed in ref. 8). The ultrasound production in these species presumably evolved independently after acquisition of the ear, because sound-producing organs differ remarkably in structure even within the same family, in which ears of the same anatomy are found on the same part of the body (1, 7, 8). In ultrasound-producing moths examined to date, the level of sounds, produced mostly in a sexual context, is high without exception [≥80 dB sound pressure level (SPL) at 1 cm] [supporting information (SI) Table S1] despite the large variation (<5 cm to >10 m) in communication distances (8). In a previous study, however, we found unusual ultrasonic communication in the Asian corn borer moth, *Ostrinia furnacalis* (Lepidoptera: Crambidae); males of this moth emit apparently weak ultrasonic courtship signals in close proximity (< 2 cm) to a female, which significantly increase the mating success of the courting male (9). The present study of this "unusual" communication has unveiled the hitherto unnoticed world of private
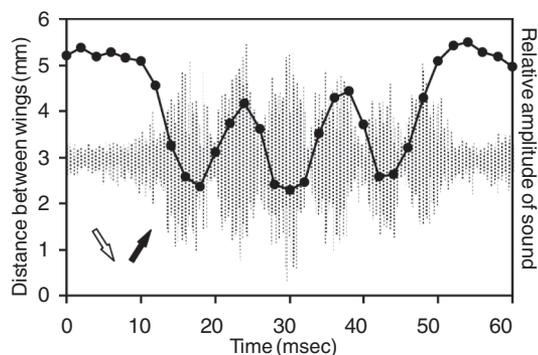
communication using quiet ultrasound. Here, we report that (*i*) ultrasonic signals of *O. furnacalis* are produced by a novel mechanism, i.e., stridulation of specialized scales on the wing and thorax; (*ii*) the signals expedite mating by suppressing the escape behavior of females during courtship; and (*iii*) the signals are extremely low-intensity, being exclusively adapted for private communication.

## Results and Discussion

**Male Ultrasound Production.** Recordings of courtship behavior and emitted ultrasound (via an ultrasound detector) indicated that sound production by males of *O. furnacalis* is associated with specific vibrations of uprightly raised wings (Movie S1). To verify that the vibrating wing generates the ultrasound, we examined the synchronicity between wing motion and the emission of ultrasonic pulses (Movie S2). Fig. 1 shows that the pattern of pulse generation clearly corresponds with up and down strokes of forewings: The first, third, and fifth pulses coincided with the upstrokes, and the second, fourth, and sixth pulses coincided with the downstrokes. Moreover, no contact between the right
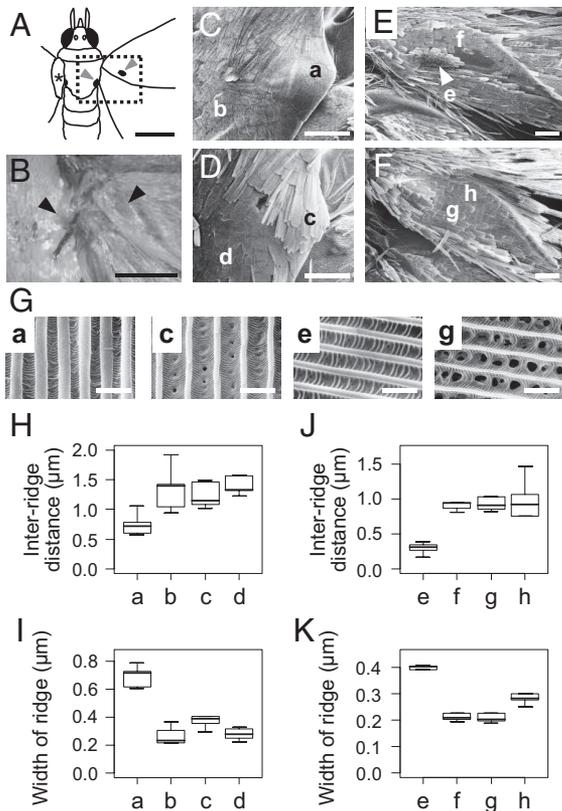
**Fig. 2.** Sex-specific scales on forewings and mesothoraxes. (*A*) Drawing of a male moth showing the positions of sex-specific scales (indicated by arrowheads) on the right of the notum (dorsal plate of mesothorax) and basal part of the right forewing. The left tegula (indicated by an asterisk) is shown intact, but the right tegula is removed to show the male-specific scales. (Scale bar: 2 mm.) (*B*) Photograph showing areas bearing the male-specific scales (indicated by a dotted box in *A*). Left and right arrowheads indicate the male-specific scales on the mesothorax and forewing, respectively. (Scale bar: 1 mm.) (*C*) Scanning electron micrograph of the right mesothorax of a male with the sex-specific scales (a) and ordinary scales (b). (*D*) The female mesothorax with scales corresponding to the male-specific scales (c) and ordinary scales (d). (*E*) The right forewing of a male with the sex-specific scales (e) and ordinary scales (f). A scaleless area is found adjacent to the sound scales. (*F*) The female forewing with scales corresponding to the male-specific scales (g) and ordinary scales (h). (Scale bars: *C–F*, 200 μm.) (*G*) Surface ultrastructure of individual scales: *a*, male-specific mesothoracic scale; *c*, female mesothoracic scale; *e*, male-specific forewing scale; *g*, female forewing scale. (Scale bar: 2 μm.) (*H–K*) Morphology of ridges on the male-specific scales and ordinary scales. Box-and-whisker plots show the median, lower and upper quartiles, and adjacent values within 1.5 × interquartile ranges from the quartiles. (*H*) The interridge distance is significantly shorter in the mesothoracic sound scale *a* than in ordinary scales *b* ($t = 4.65$, $P < 0.001$), *c* ($t = 5.32$, $P < 0.001$), and *d* ($t = 5.08$, $P < 0.001$) (Dunnett's test, $n = 5$). (*I*) The ridge is significantly thicker in scale *a* than in scale *b* ($t = 8.79$, $P < 0.0001$), *c* ($t = 8.24$, $P < 0.0001$), and *d* ($t = 4.97$, $P < 0.001$) ($n = 5$). (*J*) The interridge distance is significantly shorter in the forewing male-specific scale *e* than in ordinary scales *f* ($t = 3.71$, $P < 0.01$), *g* ($t = 3.06$, $P < 0.05$), and *h* ($t = 4.11$, $P < 0.01$) ($n = 5$). (*K*) The ridge is significantly thicker in scale *e* than scales *f* ($t = 11.00$, $P < 0.0001$), *g* ($t = 8.30$, $P < 0.0001$), and *h* ($t = 10.70$, $P < 0.0001$) ($n = 5$).

and left wings, which can produce sounds by percussion/clapping, was observed. Thus, it is evident that wing strokes are involved in ultrasound production. Given the correspondence between strokes of the wings and the sound pulses, the wing beat rate should be equal to half of the pulse repetition rate (PRR). The wing beat rate during sound production estimated from the PRR, 75.8 ± 10.7 Hz (average ± SD, $n = 60$), agreed
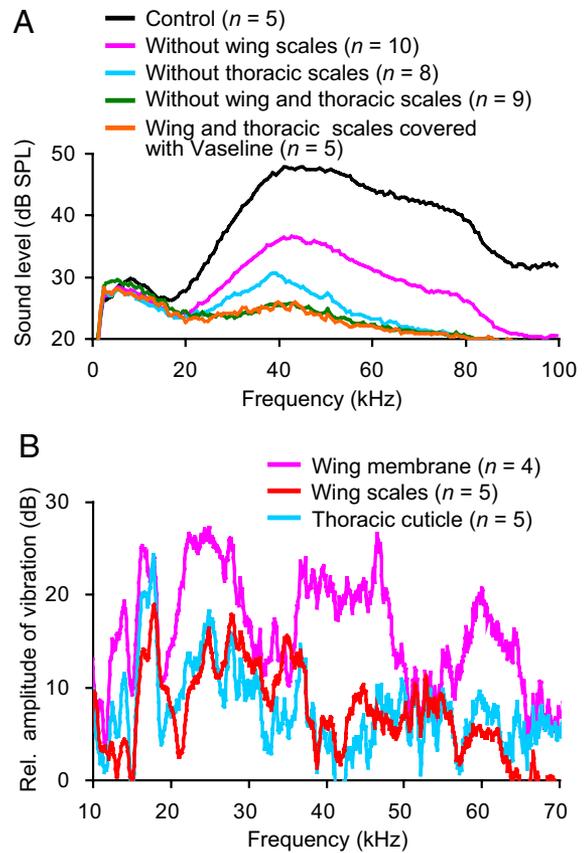


**Fig. 3.** Effects of sound scale treatments on sound production. (*A*) Sound level (dB SPL at 1 cm) diminished when either or both of the male-specific scales on the mesothorax and forewing were ablated or covered with Vaseline. $n$ = numbers of males tested. (*B*) Vibration characteristics of the male-specific scales and the cuticle underlying them measured using a laser Doppler vibrometer. Relative vibration amplitudes (dB) of objects in response to excitation by pulsed sound sweeps (10–70 kHz) are shown. The amplitude spectrum of each object was normalized to that of thoracic male-specific scales, which exhibited the weakest response among the objects examined.

well with a direct estimate from wing movement (75.9 Hz, $n = 2$). The wing beat rate during sound production was considerably higher than the rate during flying, 42.7 ± 4.2 Hz ($n = 5$, males).

Subsequently, we sought the sound-producing organs. Hitherto unreported organs were assumed to occur somewhere on the wings and/or thorax and come into contact in association with wing strokes. Indeed, we found that males possess sex-specific scales adhering to the notum (dorsal plate of mesothorax) and on the proximal part of forewings (Fig. 2 *A–G*). On the forewings of males, we also found a small scaleless membranous area adjacent to the male-specific scales (Fig. 2*E*). The sex-specific scales on the mesothorax and forewings are hidden under the tegulae when the male is in a stationary position (Fig. 2*A*). Scanning electron micrographs revealed that the male-specific scales are structurally different from ordinary scales (Fig. 2 *C–G*). The longitudinal ridges of the male-specific scales on the thorax and wings were thicker and more narrowly spaced than those of ordinary scales (Fig. 2 *H–K*). None of the sound-producing organs found in other moths, such as tymbals (vibrating blisters of cuticle), stridulatory organs, or wing castanets (8, 10), were present in *O. furnacalis*.
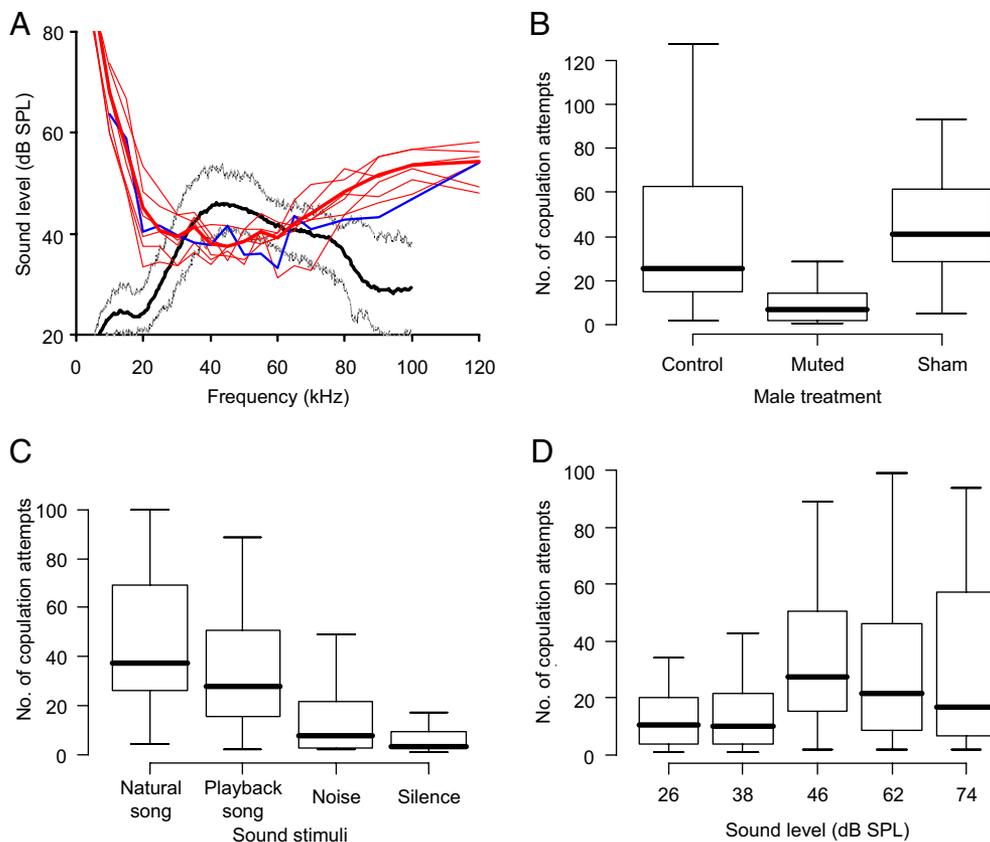
Ablation experiments clearly demonstrated that both wing-

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**Fig. 4.** Physiological and behavioral responses of moths. (*A*) Hearing threshold curves and sound level spectra of male songs. Red thin lines denote hearing threshold curves obtained from tympanal nerves of six females, and the red bold line shows the average. Blue line shows a hearing threshold curve of a male. Black bold line shows an average power spectrum of 20 male songs, and black dotted lines show the range. The songs were recorded at a distance of 1 cm, which corresponds to the distance between a female and a courting male. (*B–D*) Effect of male songs on the number of copulation attempts a courting male with damaged genital claspers can repeat before the female escapes. Males with damaged genital claspers cannot copulate and repeat copulation attempts over and over. The number of copulation attempts thus reflects the male's success in suppressing the escape behavior of the female. (*B*) Effect of male song (Control, no treatment on the sound scales; Muted, sound scales covered with Vaseline; Sham, ordinary scales covered with Vaseline). A significant difference in the number of copulation attempts is found among the male treatments [generalized linear model (GLM), errors: negative binomial; link: log, ANOVA $\chi^2_{2,57}$ = 24.1, $P <$ 0.0001]. (*C*) Effect of different sound stimuli (Natural song, the song produced by sham-operated males; Playback song, playback of recorded male songs at original sound level, ≈46 dB SPL; Noise, playback of recorded background noises; Silence, no sound broadcast). Males in the "Natural song" test group were sham-operated, and males in the other test groups were muted by covering the sound scales with Vaseline. In addition, all males were deafened to eliminate the influence of sound stimuli on their behavior. A significant difference in the number of copulation attempts is found among the sound stimuli groups (GLM, ANOVA $\chi^2_{3,76}$ = 23.6, $P <$ 0.0001). (*D*) Effect of sound level. All males were muted and deafened. Male song playbacks with different sound intensities (26, 38, 46, 62, and 74 dB SPL) were broadcast to the females. A significant difference in the number of copulation attempts is found among the sound level groups (GLM, ANOVA $\chi^2_{4,95}$ = 17.8, $P <$ 0.0014).

and thoracic scales are necessary for producing sounds. The ablation of male-specific wing scales and thoracic scales reduced the level of the ultrasound from 47.8 dB SPL (control level) to 36.6 dB SPL and 30.7 dB SPL, respectively (Fig. 3*A*). The ablation or covering of both wing and thoracic scales reduced the level to 26.0 dB SPL (background noise = 22.4 dB SPL). Although, in moths, scales on the body have been implicated in the production of ultrasounds based on behavioral observations (8, 11), we are unaware of other experimental evidence. When the wings are in the upright position for sound production (Movie S1), the direction of the longitudinal ridges of the male-specific scales on the thorax almost parallels that on the wings (Fig. 2*G*) such that scale rubbing results in maximum friction. These findings suggest that the sound-generating mechanism is friction between the male-specific scales ("sound scales") during wing vibration.

To clarify the mechanical vibrational properties of the sound scales and wing membrane underlying the scales in terms of sound radiation, vibration amplitudes of these objects under

excitation by synthetic sounds were measured by using a laser Doppler vibrometer (10). The wing membrane showed high vibration amplitudes at frequencies of ≈20–30, 40–50, and 60 kHz, whereas the wing scales and notum showed consistently lower vibration amplitudes (Fig. 3*B*). High vibration amplitudes of the wing membrane at 40–50 kHz correspond with dominant frequencies of the male songs (Fig. 3*A*), suggesting that the wing membrane underlying the scales can work as an ultrasound resonator (radiator).

The friction energy generated by the rubbing of sound scales can be amplified and radiated by the resonating wing membrane; however, it is not surprising that the sound level is low because of a lack of common stridulatory structures, e.g., steep files for efficient energy storage and air sacs for further amplification (1, 12). The mean sound level of the male song, 46 dB SPL at a distance of 1 cm, is the lowest among the ultrasound-producing moths reported to date (Table S1).

Our study adds "ultrasound production" to the list of multimodal functions of lepidopteran scales for communication. In

some species, the scales function as auditory organs receiving an acoustic signal (13) and as scent organs releasing olfactory signals (14, 15). The scales also provide visual signals based on color patterns (1, 15).

**Female Response to Male Ultrasound.** The auditory threshold curve showed a steep increase <20 kHz and a gradual rise >60 kHz (Fig. 4A), as in the congener *O. nubilalis* (16). No clear difference in the threshold curves between the sexes was observed. The threshold curves and the spectrogram of maximum amplitude of the song overlapped in the range of ≈35 to 60 kHz, showing a match between the tuning of hearing and singing. Thus, it is indicated that both males and females are capable of hearing the song during courtship because the distance between the sound-producing organs of the singing male and tympanal ears of the listening female is ≈1 cm, at which distance the sound pressure level was measured. Nevertheless, because the average sound pressure is only 8 dB above the hearing threshold (lowest threshold was 38 dB SPL at 40–45 kHz), moths at a slightly longer distance, estimated to be >2.75 cm based on the attenuation rate of sound pressure, would not be able to detect the sounds. Thus, the song is truly private, precluding the possibility of eavesdropping by conspecific rivals or natural enemies.

Behavioral experiments, using females and treated males, clarified the mode of action of the male song in *O. furnacalis*. Most of the females (>90%) readily accepted singing (intact and sham-operated) males, whereas 35% of the females rejected muted males (Table S2). To exclude the effects of variation in the males' skill to copulate smoothly, which confound the analysis of the effect of sound, males with damaged genital claspers, which repeat copulation attempts in vain, were used in the subsequent behavioral experiments. The number of copulation attempts a male could repeat before the female's escape behavior (flying away) was significantly reduced in the muted males compared with control males (Steel–Dwass test, $t = 3.5$, $P < 0.01$) and sham-operated males ($t = 4.5$, $P < 0.001$), whereas no significant difference was found between sham-operated and control males ($t = 1.3$, $P = 0.41$) (Fig. 4B). These results demonstrate that the male songs suppress the escape behavior, or locomotion in general, of the female. The effect of playback sounds was examined in subsequent experiments. The broadcast song was as effective as the natural song in suppressing escape behavior (Fig. 4C) (Steel test, $\rho = 0.5$, $t = 1.7$, $P = 0.20$), whereas playback of background noise ($t = 3.8$, $P < 0.001$) and the silent control ($t = 4.7$, $P < 0.001$) had no effect on females. The effect of sound level (26, 38, 46, 68, and 74 dB SPL) was examined next (Fig. 4D). Among the sound levels tested, 26 dB SPL was completely below the hearing threshold (Fig. 4A). The response at natural sound level (46 dB SPL) was significantly different from that at lower sound levels (26 and 38 dB SPL; Steel test, $\rho = 0.5$, $t = 2.86$, $P = 0.016$; $t = 2.59$, $P = 0.034$, respectively), confirming that songs of low sound level (46 dB SPL) are sufficient to suppress the escape behavior of females during courtship. Interestingly, a further increase in sound level neither increased nor decreased the suppressive effect of the sound (68 and 74 dB SPL; $t = 0.58$, $P = 0.94$; $t = 0.47$, $P = 0.97$, respectively). Taken together, the behavioral experiments indicate that the males produce "hushed" ultrasonic songs to suppress the female's escape behavior during courtship. The female's response to the ultrasonic songs might be cognate to the freezing behavior, which is induced in response to the echolocation calls of bats as a defense maneuver (1, 7, 17, 18). This raises the intriguing possibility that the moths have exploited both the sensory apparatus and the behavioral reaction they "inherited" from their interaction with bats. Thus, the ultrasonic sexual communication in *O. furnacalis* should prove an excellent system for testing the receiver bias model of signal evolution (1, 7).

*O. furnacalis* could have exploited low frequency sounds (possibly <1 kHz) produced from wing beats of 75.8 Hz, instead of ultrasonic courtship songs, as described in a few moths (8, 19). However, our behavioral experiments showed that the low frequency sounds from wing beats of muted males had no significant effect on the female (Fig. 4B), indicating that low frequency sounds are not involved in close-range communication in *O. furnacalis*.

To obtain a rough idea of whether the production of "quiet" ultrasound is rare in moths, we examined several moth species near at hand. It turned out that all of the pyraloid, geometrid, and noctuid moths examined produce low-intensity (< 60 dB SPL at 1 cm) ultrasound during courtship. We predict that private communication, using low-intensity ultrasound, will be discovered in many moths and other insect species for several reasons. First, the production of quiet ultrasound is likely to be low in energy costs. Reductions in energy costs would be further facilitated by the temporal pattern of sound production (4, 20), i.e., the discontinuous emission of pulses with intervals (Movie S2) and short duration of the song (≈1.5 s) (9). Second, quiet ultrasound production is likely to be facilitated by simple organs such as specialized scales (Fig. 2 C–G) and thus may evolve easily compared with elaborate organs requiring major modifications of the body surface (e.g., tymbals) (10). Finally, as already mentioned, quiet ultrasonic songs reduce the risk of interception by conspecific competitors, predators like insectivorous bats (1, 3, 7, 8) and perhaps parasitoids (2). In moths, competition among males is strong, because many males are likely to be attracted around a pheromone-releasing female (1, 14). Thus, the quietness of courtship songs would be crucial to avoid eavesdropping by neighboring males. Also predatory bats would not be able to detect *Ostrinia* songs from a distance >20 cm, assuming bats' auditory threshold to be 20 dB SPL (21). Our discovery of extremely low-intensity ultrasonic communication may point to a whole undiscovered world of private communication using quiet ultrasound.

## Materials and Methods

**Experimental Animals.** Larvae of *O. furnacalis* in corn stems were collected at Akiruno, Tokyo, Japan (35.4°N, 139.2°E) during December 2005 to March 2006. Cultures of *O. furnacalis* were maintained in the laboratory as described in ref. 9.

**Scanning Electron Microscopy.** A newly emerged moth was killed with the vapor of ethyl acetate and mounted on a coverslip with an instantaneous adhesive. After drying in the air, the sample was coated with platinum, using a sputter coater (E-1030; Hitachi), and observed with a scanning electron microscope (S-2000; Hitachi) at 10 kV.

**Treatment of Scales.** For mute treatment, 1-day-old males were anesthetized with $CO_2$. The thoracic tegulae of both sides, which hide the male-specific scales on the mesothorax and forewings, were removed using forceps under a stereomicroscope. The male-specific scales on the mesothorax and/or bases of forewings were then removed with a fine pin or covered with a small amount of Vaseline.

**Sound Recording.** Single untreated or treated males were confined with 10 virgin females in a cubic mesh cage (18 × 18 × 18 cm), which was placed in a one-side opened soundproof box (40 × 40 × 70 cm). The male courtship songs were recorded with a 1/4 inch condenser microphone [type 4939; Brüel & Kjær] connected to pre- and conditioning-amplifiers (type 2670 and 2690 with a 0.02–100 kHz band-pass filter; Brüel & Kjær) at a distance of ≈1 cm from the singing male, which corresponds to the distance between the male and female during acoustic communication. The acoustic signals were digitized by an analog/digital (A/D) converter (Wavebook 512A IOtech) at a sampling rate of 300 kHz (14 bits) and high pass-filtered (>10 kHz). Power spectra were computed by using a Hanning window with an FFT size of 512 points. Relative sound amplitudes were converted to sound pressures in dB peSPL (peak equivalent sound pressure level in decibels relative to 20 μPa rms, abbreviated as dB SPL for brevity), using a sound level calibrator (type 4231, Brüel & Kjær; 94 dB SPL at 1 kHz).

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**Simultaneous Recordings of Wing Motion and Ultrasound.** The wing movements and ultrasonic songs of a singing male were recorded synchronously. During the late scotophase, males and females were introduced into a cylindrical mesh cage (diam. 75 × 75 mm). We recorded courtship behavior of the moths under red dim light, using a 300,000-pixel ultrahigh-speed, high-sensitivity camera and its data acquisition system (Japan Broadcasting Corporation). Simultaneously, male songs were recorded by the Brüel & Kjær microphone and the Wavebook A/D converter systems (see *Sound Recording*) that were synchronized with the high-speed camera system by means of a beam trigger (Movie S2). The motions of a singing male were captured at a rate of 500 frames per second for 288 ms. To trace wing movements (up and down strokes), we measured the distance between the tips of the right and left forewings with Scion Image software. The changes in the distance were superimposed on the oscillogram of recorded ultrasonic pulses. The wing beat rate during sound production was estimated from the wing movements and from the rate of repetition of the ultrasonic pulses. In addition, the rate during flying was estimated from the wing beat sound of tethered males.

**Mechanical Vibration of Sound-Producing Organs.** Mechanical vibrational properties of male-specific scales (sound scales) and the cuticle under the sound scales were investigated. The tegulae were removed from a freshly killed moth (1-day-old male) to expose the sound-producing organs. For measuring vibrations of the cuticle under the sound scales, all of the sound scales on the wing or those on the thorax were removed, as described in *Treatment of Scales*. The moth with fore- and hind wings in a horizontal position was mounted on a plastic holder with the wing tips attached to the holder with an instantaneous adhesive. The sound-producing organs were driven by sound stimuli of 10 ms (rise/fall time 1 ms, swept linearly from 1 to 100 kHz) generated every second by a function generator (type 5061 with a sampling rate of 10 MHz; Tabor Electronics). The sound stimuli were broadcast through an ultrasound loudspeaker (model S56; UltraSound Advice; a flat frequency response in 5–50 kHz and a shallow drop of >10 dB in 50–80 kHz) via an amplifier (model S55; *, UltraSound Advice; frequency range 18–300 kHz ± 3 dB). The distance from the speaker to the mounted moth was ≈20 cm. The level of the sound stimulus at the moth was ≈80 dB SPL when measured by the Brüel & Kjær microphone systems described above.

Vibration amplitudes were measured as velocity with a laser Doppler vibrometer (LV-1720 Ono Sokki), and monitored on an oscilloscope (WaveRunner; Iwatsu). The laser beam (≈20 μm in diameter) was scanned over the surface of the organs to locate the area with maximal vibration. The vibration amplitudes of the organs in response to 100 stimuli were averaged. Vibration velocity spectra were normalized to the sound energy using AutoSignal software (Systat) and converted to displacement. Displacement amplitudes exceeded background in the range 10–70 kHz. The vibration amplitudes of the wing and thoracic sound scales were obtained by subtracting the amplitudes of the wing and thoracic cuticles from those of intact wing and thorax, respectively. The amplitudes of objects were normalized to that of thoracic sound scales, which exhibited the weakest response among the objects examined and displayed on a relative dB scale for comparison (Fig. 3*B*).

**Electrophysiological Recording from Tympanal Nerves.** In *Ostrinia*, a pair of tympanal nerves run through a branch of the first abdominal ganglion and the abdominal connective to a thoracic ganglion (16). The dorsal portion of the thorax was removed to expose the tympanal branch as described in ref.22 with slight modification. Either the left or right tympanal branch was hooked onto a recording tungsten electrode, and then an indifferent silver electrode was positioned in the flight muscle of the thorax. The nerve was covered with a 1:1 mixture of Vaseline and paraffin oil during recording to avoid desiccation. A Technics tweeter (EAS10TH400B) was placed 30 cm from the moth facing the insect's ear. Tympanal nerve activity was band-pass filtered, amplified by a custom-built amplifier, and displayed on an oscilloscope through an audio monitor. The sound pressure threshold was defined as the pressure level

necessary to elicit 1–2 spikes in at least eight out of 10 stimulations. Hearing sensitivity was tested in 5–10 kHz steps at between 5 and 120 kHz. The frequencies were tested in random order.

**Behavioral Experiments.** Two-day-old virgin males and 2- to 4-day-old virgin females were used throughout the behavioral experiments. The experiments were carried out by using a laboratory flight tunnel (diameter 0.3 × 2 m, wind speed of 0.1 m·s$^{-1}$) under red dim light (0.2 lux) and at 24°C in the last 2 hours of the scotophase of a 16 h:8 h light:dark cycle, when moths show active mating behavior (23). For the mute-operation, sound scales on the mesothorax and forewings were covered with Vaseline (see *Treatment of Scales*). For the sham-operation, ordinary scales, instead of the sound scales, on the mesothorax and forewings were covered with Vaseline. The deaf-operation was carried out by puncturing the tympanal membrane with a sharp pin (9). All treatments were performed on $CO_2$-anesthetized moths under a stereomicroscope one day before the experiments. A single male was introduced into the flight tunnel housing five to 10 females, and the courtship behavior of the male was observed as described in ref. 9. The emission of ultrasound was continuously monitored with an ultrasound detector (Model D240x; Pettersson Elektronik AB). At the end of each test, the pair of moths observed was removed from the tunnel to exclude pseudoreplication.

*Experiment 1.* The significance of male song production during courtship was verified using muted males. The acceptance (copulation) or rejection (escape) responses of a female to nonsinging (muted) and singing (control and sham-operated) males were compared.

*Experiment 2.* The effect of male songs on the female's escape behavior was examined using muted, sham-operated, and control males, all with damaged claspers. Males were disabled from copulating by damaging their genital claspers using tweezers, and a male's success in suppressing the female's escape behavior was evaluated by the number of copulation attempts the male could repeat before her escape.

*Experiment 3.* Experiments were carried out to confirm that suppression of the female's escape behavior is caused by the sounds produced by the male. Muted or sham-operated males, with damaged genital claspers, were further deafened so as to eliminate the influence of sound stimuli on their behavior. The broadcast stimuli were, playbacks of male songs at the original sound level (≈46 dB SPL at 1 cm), playbacks of background noises, and no sound. Twenty male songs recorded, using the Brüel & Kjær microphone system, were edited by BatSound software to include one group of pulses and a silent interval (58–176 ms in total as recorded duration). The edited data were transmitted to a Tabor Electronics function generator with a sampling rate of 300 kHz (14 bits), using the software ARB-SOFT (Toyo). The sound stimuli, continuously looped back by the function generator, were broadcast to a pair of moths during courtship from a loud speaker (UltraSound Advice) set 20 cm from the pair (*n* = 20). Before each test session, we recorded the broadcast sound stimuli with the Brüel & Kjær microphone system to ensure that the amplitude, frequency, and temporal characteristics were as intended.

*Experiment 4.* The effect of sound level on the suppression of female escape behavior was examined using the same experimental setup as in the previous experiment. Male songs were played back to 20 pairs of courting moths each at sound levels of 26, 38, 46, 62, and 74 dB SPL. The sound used in each test was randomly chosen from the 20 edited songs, and the level and other characteristics of the sound were checked as in the previous experiment.

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Nakano *et al.*