Universidade
Estadual de Londrina

PARANÁ
GOVERNO DO ESTADO

# CENTRO DE CIÊNCIAS BIOLÓGICAS
# PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

## Edital Complementar nº 06 /2025

## Torna público o artigo científico que servirá de base para a Etapa I da Prova Escrita da seleção de aluno Regular de Mestrado – oferta 2026-1.

O Programa de Pós-Graduação em Ciências Biológicas da Universidade Estadual de Londrina (PPG-CB/UEL), em conformidade com o disposto no Edital nº 03/2025 - Oferta aluno regular 2026/1, que estabelece normas e procedimentos de inscrição e seleção para o ingresso no Programa de Pós-Graduação Stricto sensu em Ciências Biológicas, na condição de Estudante Regular, torna público o artigo científico que servirá de base para a Prova Escrita (Etapa I), conforme estabelecido no item 7.2., subitem 7.2.3 do referido edital.

**Artigo científico:** Kawahara, A. Y., & Barber, J. R. (2015). Tempo and mode of antibat ultrasound production and sonar jamming in the diverse hawkmoth radiation. *Proceedings of the National Academy of Sciences*, 112(20), 6407-6412. O artigo completo pode ser consultado no Anexo 1 do presente edital ou por meio de download direto em https://www.pnas.org/doi/10.1073/pnas.1416679112 ou https://doi.org/10.1073/pnas.1416679112

Permanecem inalteradas as demais disposições do Edital nº 03/2025.

Londrina, 24 de setembro de 2025.

**Profa. Dra. Ana Paula Vidotto Magnoni**
Coordenadora
Programa de Pós-graduação em Ciências Biológicas
Universidade Estadual de Londrina

# Anexo 1 – Artigo científico

# Tempo and mode of antibat ultrasound production and sonar jamming in the diverse hawkmoth radiation

**Akito Y. Kawahara**[a,1,2] **and Jesse R. Barber**[b,1,2]

[a]Florida Museum of Natural History, University of Florida, Gainesville, FL 32611; and [b]Department of Biological Sciences, Boise State University, Boise, ID 83725

Edited by May R. Berenbaum, University of Illinois at Urbana–Champaign, Urbana, IL, and approved January 5, 2015 (received for review August 29, 2014)

**The bat–moth arms race has existed for over 60 million y, with moths evolving ultrasonically sensitive ears and ultrasound-producing organs to combat bat predation. The evolution of these defenses has never been thoroughly examined because of limitations in simultaneously conducting behavioral and phylogenetic analyses across an entire group. Hawkmoths include >1,500 species worldwide, some of which produce ultrasound using genital stridulatory structures. However, the function and evolution of this behavior remain largely unknown. We built a comprehensive behavioral dataset of hawkmoth hearing and ultrasonic reply to sonar attack using high-throughput field assays. Nearly half of the species tested (57 of 124 species) produced ultrasound to tactile stimulation or playback of bat echolocation attack. To test the function of ultrasound, we pitted big brown bats (*Eptesicus fuscus*) against hawkmoths over multiple nights and show that hawkmoths jam bat sonar. Ultrasound production was immediately and consistently effective at thwarting attack and bats regularly performed catching behavior without capturing moths. We also constructed a fossil-calibrated, multigene phylogeny to study the evolutionary history and divergence times of these antibat strategies across the entire family. We show that ultrasound production arose in multiple groups, starting in the late Oligocene (~26 Ma) after the emergence of insectivorous bats. Sonar jamming and bat-detecting ears arose twice, independently, in the Miocene (18–14 Ma) either from earless hawkmoths that produced ultrasound in response to physical contact only, or from species that did not respond to touch or bat echolocation attack.**

acoustic | antipredator defense | bat–moth interactions | evolution | Sphingidae

Bats and moths have dominated the night sky for more than 60 million y (1, 2). The shared evolutionary history between these diverse groups has armed bats with effective attack behaviors and moths with powerful counter measures (3). Of the nearly 140,000 moth species (4), more than half may possess ultrasonically sensitive bat-detecting ears (5). Moth ears are connected to neuronal circuits that steer the animals away from bats at low sonar call intensities, trigger aerobatic evasive behaviors—such as directed turns, loops, spirals, and power dives (3)—and elicit ultrasound production at high sonar intensities (6–8). Ultrasonic ears are known to have independently evolved at least 18 times in seven insect orders (9) and possibly 10 or more times in Lepidoptera (5).

Recent work on bat–moth aerial encounters has focused on tiger moths (Arctiinae; reviewed in ref. 3, but also see ref. 10). Tiger moths can hear bat echolocation and have paired metathoracic tymbals that beam ultrasonic clicks back at bats (6, 7). The sounds that these moths make can startle bats (11), warn them of bad taste (12, 13), and jam bat sonar (14–16). Jamming likely functions to confuse the bat because it interferes with the bat's perception of its own echoes that are reflecting off of its prey (16, 17). The evolution of moth antibat ultrasound production is thought to have originated from simple warning sounds and developed into complex signals that jam bat sonar (14). However, moth ultrasound production, one of the most sophisticated responses to bat attack (3), has never been examined in a phylogenetic context.

Hawkmoths (family Sphingidae) are among the most conspicuous nocturnal Lepidoptera (18); they are generally long-lived, strong flying pollinators (19, 20). Because of their large size and lack of known adult toxins (21, 22), sphingids are important prey for bats (23–28). Some hawkmoths possess specialized sound-receiving structures on the head, comprised of the labral pilifers and palps that are sensitive to the ultrasonic frequencies of bat sonar (29, 30). One group of hawkmoths, the Choerocampina, has been shown to produce ultrasound by stridulating modified genital valves when stimulated by playback of bat sonar attack (6). However, the function and taxonomic breadth of ultrasound production in hawkmoths is unknown.

To determine the function of ultrasound production, we conducted bat–moth interaction experiments in the laboratory. We predicted that the pattern over time of a naïve bat's success in catching stridulating hawkmoths would differentiate the three hypotheses for their function (12). If hawkmoths are jamming bat sonar, the moth sounds should be immediately and consistently effective at thwarting attack. Alternatively, if the moth sounds startle bats, the predators should initially be deterred but habituate over time. If moths are warning bats of distasteful toxins, bats should initially capture and drop moths, then learn to avoid them (12, 13). We paired these laboratory experiments with high-throughput behavioral field assays and used genetic

## Significance

Ultrasound production is one of the most sophisticated antibat strategies in nocturnal insects, yet it has never been thoroughly studied in a phylogenetic framework. We conducted high-throughput field assays using playback of echolocation attack sequences, laboratory bat–moth interaction experiments, and fossil-calibrated phylogenetic analyses to provide the first evidence that multiple unrelated hawkmoth species produce ultrasound and jam bat echolocation. Our robust tree demonstrates that sonar jamming evolved twice during the Miocene after the radiation of insectivorous bats. We provide an example of the power behind collaborative science for revealing the function and historic pattern of behavior, and predict that ultrasound production is a widespread antibat strategy in the extraordinary diversity of nocturnal insects.

---

Author contributions: A.Y.K. and J.R.B. designed research, performed research, contributed new reagents/analytic tools, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. KP719983–KP720300). Datasets and accompanying files are available from the Dryad Data Repository (www.datadryad.org; accession no. 10.5061/dryad.3450r).

[1] A.Y.K. and J.R.B. contributed equally to this work.

[2] To whom correspondence may be addressed. Email: kawahara@flmnh.ufl.edu or jessebarber@boisestate.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1416679112/-/DCSupplemental.

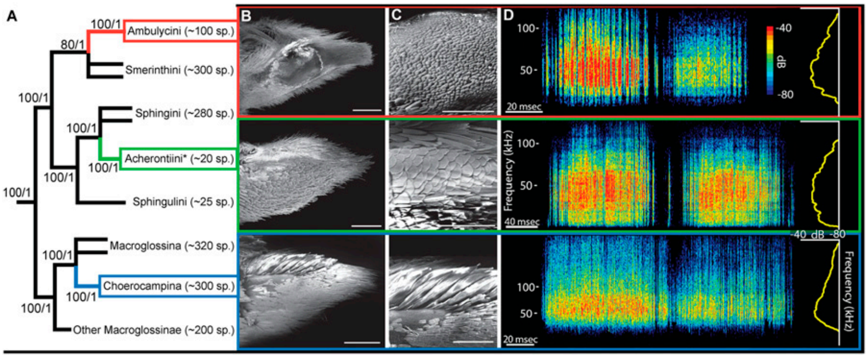

**Fig. 1.** Antibat ultrasound in three hawkmoth lineages. (*A*) ML phylogenetic tree of Sphingidae simplified to show relationships among major groups. Numbers by branches are ML bootstrap support values and Bayesian posterior probabilities; numbers after each taxon name indicate the known species diversity according to hawkmoth classification (18). (*B*) Lateral view of the left male genital valve showing ultrasound-producing scales, anterior to the left. (Scale bar, 1 mm.) (*C*) Close-up view of stridulatory scales, anterior to the left. (Scale bar, 500 μm.) (*D*) Spectrograms (frequency in kilohertz on the *y* axis and time in milliseconds on the *x* axis; FFT 256) and power spectra (frequency in kilohertz on the *y* axis and sound intensity in decibels on the *x* axis; FFT 256) of modulation cycles produced by species from each of the three lineages; (*Top* to *Bottom*) *Ambulyx pryeri* (Ambulycini), *Psilogramma discistriga* (Acherontiini), *Xylophanes falco* (Choerocampina). In all cases, two bursts of sound are created as the valves scrape dorsomedially across the abdominal file, then after a short pause the valves scrape ventrolaterally across the file as they return to a resting position. See Movies S1–S3 for structural movement of valves. **Psilogramma* is here treated as part of the broader Acherontiini.

material from the same moths to build a fossil-calibrated, multigene molecular phylogeny of hawkmoths. Our behavioral experiments and phylogenetic analyses illustrate, for the first time to our knowledge, the function and evolution of antibat ultrasound production in a diverse group of insects.

## Results and Discussion

We present a comprehensive behavioral dataset and couple it with the most complete and robust phylogeny of hawkmoths to date, to reveal a fascinating history of bat–moth interactions. After testing the ultrasonic response of 124 hawkmoth species to tactile handling and playback of multiple frequency-modulated bat echolocation attack recordings (Dataset S1), we found that males of nearly all species in the Acherontiini *s.l.*, Ambulycini, and Macroglossinae generated broadband, primarily ultrasonic sounds with their genitalia (Fig. 1 *B*–*D*). Surprisingly, all three groups have a strikingly different modified scale patch on the inner surface of their genital valves and the stridulation mechanism is unique to each (Movies S1–S3). Some of these ultrasound-producing species lack ears (e.g., Ambulycini, Dilophonotini, and lower Macroglossini), implying that ears are not a prerequisite for sound production. Nearly all species in the Acherontiini *s.l.* and Choerocampina produced an ultrasonic reply to bat echolocation, confirming the pioneering work of Roeder et al. (29) and Göpfert and Wasserthal (30). Hawkmoths that have ears did not respond acoustically to echolocation when their palps were removed, a result consistent with Roeder and Treat (31) and Roeder et al. (32), who showed that the acoustic sensitivity of Choerocampina decreases dramatically after amputation of the labial palp. Some female hawkmoths also produced ultrasound (Dataset S1), but because our sampling of females was very limited, these data were consequently not incorporated into the ancestral state reconstruction analyses.

We pit big brown bats (*Eptesicus fuscus*; Vespertilionidae) against hawkmoths in two experiments to test the function of hawkmoth antibat ultrasound. In our first experiment, an adult *E. fuscus* was exposed to individuals of the falcon sphinx moth (*Xylophanes falco*) for four consecutive nights, and a second bat was similarly tested for eight nights. Recent diet analyses reveal that *E. fuscus* consistently preys on Lepidoptera from a variety of families (33), so this was an appropriate bat species for these experiments. Each night, two sound-producing, palatable *X. falco* were randomly presented along with eight other palatable, silent moths: two *X. falco* with their sound-producing genital structures ablated, two white-lined sphinx moths (*Hyles lineata*; a naturally silent hawkmoth, similar in size to *X. falco*, included as a negative control for prey body size), and four silent, palatable, female greater wax moths (*Galleria mellonella*). Logistic regression analysis showed that all three types of silent moths were significantly more likely to be captured than sound-producing *X. falco*: ablated *X. falco* were 33.0-times [95% confidence interval (CI) = 2.9–374.4] more likely to be captured, *H. lineata* were 11.0-times [95% CI = 1.4–85.1] more likely, and *G. mellonella* were 121.0-times (95% CI = 6.7–2188.4) more likely (Fig. 2*A*). The probability of capture did not significantly vary between different nights, indicating that bats did not habituate to the moths' acoustic defense and that the moth's sound production was

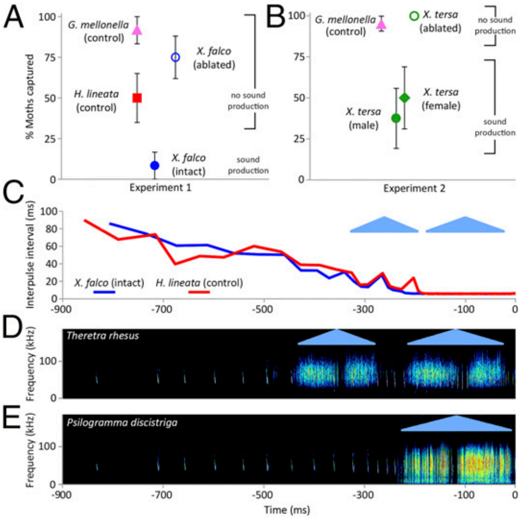

**Fig. 2.** Results of bat–moth interaction experiments. (*A*) Male *X. falco* and (*B*) male and female *X. tersa* are significantly less likely to be captured than hawkmoths with sound-producing structures removed or control moths. Ablated *X. tersa* data ($n = 4$) were collected during noncontiguous trials with the same bats. (*C*) Big brown bats (*E. fuscus*) show similar echolocation attacks, as measured by interpulse interval patterns, when attacking sound-producing and silent moths (also see Fig. S8). Spectrograms of ultrasonic response to playback of a frequency-modulated sonar attack for taxa from the two groups thought to jam bat sonar: *Theretra rhesus* (*D*) and *Psilogramma discistriga* (*E*). A blue triangle indicates a hawkmoth antibat modulation cycle.

immediately and consistently effective. Bats that failed to capture sound-producing hawkmoths often performed catching behavior without subduing prey (Movie S4).

The second experiment was conducted on naïve bats to control for potential previous experience with sound-producing prey. Each evening, for four consecutive nights, bats were exposed to two sound-producing *Xylophanes tersa* males, two sound-producing *X. tersa* females, and four silent female *G. mellonella* controls. Logistic regression analysis showed that the odds of a silent control moth being captured were 16.7-times (95% CI = 1.3–100.0) greater than the odds of an *X. tersa* male being captured and 25.0-times (95% CI = 2.1–100.0) greater than the odds of an *X. tersa* female being captured (Fig. 2*B*). As in the first experiment, hawkmoth ultrasound production (in both male and female moths) was immediately and consistently effective at thwarting attack and bats regularly performed catching behavior when failing to capture moths. We examined sonar emissions and found that bats, in both experiments, attacked all silent control and sound-producing moths with comparable echolocation patterns (Fig. 2*C*), similarly progressing through the approach, track, and terminal phases of attack (34).

Results of these multinight experiments are not congruent with either a warning or startle function and instead are consistent with a jamming role for hawkmoth ultrasound production. Two bats avoided the first sound-producing hawkmoth they experienced, suggesting that startle may function ephemerally, as has been shown in tiger moths (12). However, we view it as unlikely that short-lived startle behavior is maintaining and driving the evolution of sound production in bat–moth interactions. In addition, two points further support that hawkmoths are not warning of bad taste: (*i*) bats consumed all ablated and control hawkmoths that were captured; and (*ii*) we conducted palatability experiments on three continents with 26 species of distantly related hawkmoths and 5 different bat species, and in all cases all hawkmoths tested were completely consumed (Table S1).

The only moth previously known to definitively jam sonar, *Bertholdia trigona* (Erebidae: Arctiinae), has a duty cycle of ∼44% (14). In contrast, *Cycnia tenera*, a tiger moth that may possess limited jamming ability, has a duty cycle of ∼8% (8, 12), and the sound-producing tiger moth *Euchaetes egle*, which appears to be unable to jam sonar, has a duty cycle of only ∼3% (12). Duty cycle, or sound per unit time, is likely related to jamming efficacy (35, 36). *X. tersa* and *X. falco* have respective duty cycles of ∼18% and ∼27%. It seems likely that antibat jamming is a continuum, with higher duty cycles being more effective at confusing predators. Based on the results of our bat–moth experiments, we estimated that duty cycles of ∼20% or higher are capable of jamming bat sonar in species that we have not tested in bat–moth interactions.

We examined our behavioral findings in an evolutionary context with a new, multigene molecular phylogeny. Many relationships that were previously challenging to estimate were resolved. Our maximum-likelihood (ML) and Bayesian consensus trees were topologically near identical, resulting in well-resolved phylogenies with high branch support (≥80% bootstrap and ≥0.99 posterior probabilities for ~80% of nodes) (Figs. S1 and S2). Relationships were largely consistent with a previous five-gene hawkmoth phylogeny (37) but there were some clear differences, such as the placement of the day-flying, earless Hemarina. There was strong branch support for the monophyly of Sphingidae, subfamilies Macroglossinae and Sphinginae (with ∼1,000 and ∼300 described species, respectively). Additional notable groups and relationships with strong branch support at the tribal level include Acherontiini *s.l.*, Acherontiini *s.s.*, Ambulycini, Choerocampina, the *Psilogramma* group, and the placement of Sphingulini as the sister group to the Sphinginae. Our results are discordant with the current higher-level classification of the group (19), as the Smerinthinae (a diverse subfamily of ∼450 species) and three tribes (Dilophonotini, Macroglossini, Smerinthini) were paraphyletic with strong branch support.

Our fossil-calibrated tree reveals that the first ultrasound-producing hawkmoths arose in the late Oligocene at ∼26 Ma (CI 32–20 Ma), independently in the Ambulycini and Macroglossini (Fig. 3 and Fig. S3). Although at least six lineages seem to have gained the ability to produce ultrasound in hawkmoths, only two—the Choerocampina and Acherontiini *s.l.*—appear to jam bat sonar. These two groups originated in the Miocene (18–14 Ma), either from earless species that produced ultrasound in response to physical contact (Macroglossina) or species that did not respond to touch or bat echolocation attack (most Sphinginae) (Fig. 3 and Figs. S4–S6). The ability to jam (as determined by high duty cycle) was subsequently lost in a few lineages that diversified in the Pliocene, ∼5 Ma (Fig. S7). The Ambulycini and some non-Choerocampina Macroglossinae produce duty cycles theoretically capable of jamming bat sonar, but they lack ears and produce ultrasound only to tactile stimulation. The evolution and maintenance of ultrasound production in these groups might be driven by response to a broader spectrum of predators [i.e., ultrasonically sensitive rodents, shrews, and primates (38)], a predator-suite for which startle is perhaps more effective.

The origin of hawkmoth ultrasound production took place soon after the origin of tiger moths, the latter of which arose in the late Eocene (39). Many tiger moths are chemically defended and they use sounds to warn bats of their toxins (12, 13, 40). Hawkmoths do not appear to sequester plant toxins in their adult tissues (41), are known to be palatable to birds (42), and we have confirmed they are palatable to bats (Table S1). Although hawkmoths and tiger moths are distantly related (43–46) and have not shared a common ancestor for at least 100 million y (39), we predict that acoustic mimicry of chemically defended tiger moths (13) is a likely additional function of hawkmoth ultrasound, because both tiger moth and hawkmoth sounds are similar, the animals occupy overlapping distributions, and they appear to have close age origins. We hypothesize that even for earless hawkmoths that only produce ultrasound when handled, acoustic mimicry might be an important selective force (47), because bats likely make prey selection decisions during aerobatic handling maneuvers that can span a second or more.

An additional selective pressure that cannot be discounted is sex. Many tiger moths are known to use ultrasound for courtship (48). Although there have been no definitive empirical data confirming that hawkmoths use ultrasound during mating, Mell (49) observed, on two occasions, a male hawkmoth (*Psilogramma menephron*) stridulating while circling above a female. For several hawkmoth species tested, both sexes used their genitalia to produce ultrasound (Dataset S1). We thus predict that some hawkmoths use ultrasound during mating and that sexual selection likely plays a role in the maintenance and evolution of sound production.

In this study, we simultaneously investigated proximate and ultimate questions of animal behavior (50), via laboratory and field experiments, to begin uncovering the historic patterns of antibat strategies in a long-standing arms race. We predict that ultrasound production is widespread in the extraordinary diversity of nocturnal insects, mediated by aposematic, mimetic (both Batesian and Müllerian), and jamming mechanisms.

## Materials and Methods

**Taxon Sampling.** Hawkmoths were sampled at 70 sites in 32 countries (Dataset S2). We conducted field-based echolocation playback behavioral experiments to 124 species before storing tissues of each specimen in 100% ethanol for eventual inclusion in the phylogenetic analysis. This integrated approach enabled us to obtain behavioral and molecular data from the same specimens to better examine the evolution of antibat behaviors. Voucher specimens of all moths are stored in the collection of the Florida Museum of Natural History, University of Florida; acoustic files are archived at the Cornell Lab of Ornithology Macaulay Library (Accession no. 3884). New DNA sequence data are available from GenBank (Accession nos. KP719983-KP720300),

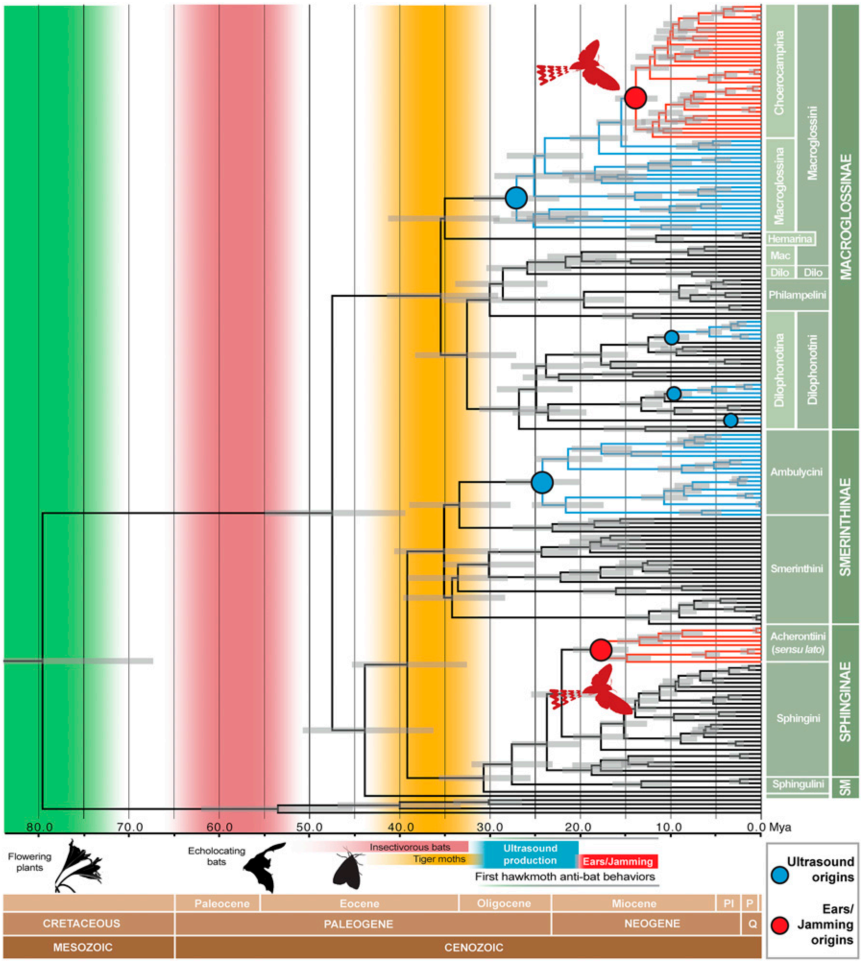

**Fig. 3.** Time-calibrated molecular phylogeny of hawkmoths showing the evolution of ears, ultrasound production, and bat sonar jamming. Branches show 95% CI (gray) from the posterior distribution of trees. Blue branches denote clades that create ultrasound in response to touch; red clades indicate groups that respond to both touch and bat echolocation attack. Blue dots indicate the origin of ultrasound production; red dots indicate the predicted origin of ears and bat sonar jamming. Outgroups are excluded from the figure.

datasets and accompanying files are available from the Dryad Data Repository (www.datadryad.org; Accession Number 10.5061/dryad.3450r).

**High-Throughput Behavioral Assays.** We tethered 734 moths in free flight at the end of a 5-mm-diameter hollow plastic rod; a monofilament line was tied between the thorax and abdomen, threaded through the rod, and held securely while the flying moth was queried for acoustic response to tactile stimulation and playback of bat attacks. We examined acoustic responses in several tethering scenarios and elected to use the above method, as it did not interfere with sound production. To record moth sounds, we used Avisoft UltraSoundGate 116Hn hardware (sampling at 250 kHz onto a laptop computer running Avisoft Recorder software) and a CM16 condenser microphone (±3 dB, 20–140 kHz) positioned 10 cm from the posterior end of the moth's abdomen (location of the sound-producing structures) and presented moths with three echolocation attack sequences from a speaker (Avisoft UltraSoundGate Player BL Pro-2, ±9 dB, 18–100 kHz) placed 10 cm from the head, perpendicular to the length of the moth body (*Supporting Information*).

We measured moth signals using Avisoft SASLab Pro and defined each cycle of the stridulatory apparatus as a modulation cycle. All signal parameters were computed from three modulation cycles per individual. We used responses from tactile trials to characterize signals to prevent corruption from overlapping bat sounds in the echolocation playback trials. To determine if moth sounds were spectrally or temporally different when produced during tactile or playback trials, we examined several modulation cycles from multiple species and found no differences. We measured temporal parameters from the oscillogram and spectral values from power spectra (FFT 1024, 50% overlap). To calculate duty cycle of the moth sounds, we counted the number of clicks that occurred in 100 ms, multiplied this by the average click duration of the modulation cycle (both measured using the Pulse Train Analysis tool in SASLab Pro), and divided this value by 100. We used this approach to allow for a direct comparison with tiger moth acoustic analyses (14, 35). Duty cycles, peak frequencies, and bandwidths (defined as the difference between the upper and lower frequency that is ±15 dB from the peak frequency) are presented in Table S2.

**Behavioral Laboratory Experiments.** We conducted all vertebrate care in accordance with Boise State University's Animal Care and Use Committee's guidelines (IACUC #006-AC11-015). We collected big brown bats (*E. fuscus*) as adults or preflight juveniles at colonies in Idaho, and maintained them on a diet of mealworms (larvae of *Tenebrio molitor*) and greater wax moths (*G. mellonella*) along with vitamin supplements. We performed experiments in an anechoic foam-lined indoor flight facility at Boise State University (7.6 m × 6.7 m × 3 m). During each experimental presentation of a flying moth tethered to a fine monofilament line, bats were allowed to hunt for 1 min. To establish a positive control, *E. fuscus* bats were trained to hunt silent, palatable, female *G. mellonella*; this hunting behavior was carefully observed to ensure that all bats used in these experiments were similar in their effectiveness at capturing moths. To capture each bat–moth interaction, we used three digital, high-speed, infrared-sensitive video cameras (Basler Scout, 120 frames per second) and Maxtraq3D software (Innovison Systems), and a desktop computer. We illuminated the interaction space with eight infrared Wildlife Engineering LED arrays and recorded ultrasound with a four-channel Avisoft UltraSoundGate 416H (sampling at 375 kHz) and four CM16 condenser microphones [±3 dB(Z), 20–140 kHz], using the Avisoft Recorder software described above. We calculated interpulse intervals (the temporal gap between sonar pulses) of echolocation attacks in Avisoft SASlab Pro.

**Molecular Dataset Construction.** The molecular dataset included five nuclear loci and one mitochondrial gene, totaling 7,449 bp, building on an available dataset of hawkmoths (37). Outgroups were selected based on previous phylogenetic analyses of Bombycoidea (51, 52). Genes and their sequence lengths were: pyrimidine biosynthesis (*CAD*; 2,928 bp), dopa-decarboxylase (*DDC*; 1,282 bp), elongation factor-1α (*EF-1α*; 1,228 bp), Period (*PER*; 951 bp), wingless (*WG*; 402 bp), and cytochrome oxidase 1 (*COI*; 658 bp). The majority of sequence data were generated at the University of Maryland, College Park, MD, using RT-PCR, for which the primers, protocols, and molecular techniques are outlined in recent publications (e.g., refs. 37, 43, 51, and 53). Individual gene datasets were initially created to test for laboratory contamination, and subsequently concatenated into a single data matrix. For further details, see *Supporting Information*.

**Alignment, Partitioning, and Phylogenetic Methods.** All genes were concatenated and aligned with MAFFT v7.037 (54). The most optimal partitioning strategy and model of evolution was identified through PartitionFinder v1.0.1 (55). ML analyses were run in RAxML v7.3.2 with 1,000 tree searches with a backbone topological constraint (56). The Bayesian analysis was run in MrBayes v3.2 (57) with one cold chain and three hot chains, also implementing a backbone constraint. Converged MrBayes runs were combined after the deletion of burn-in and a majority rule consensus tree was created. In all analyses, trees were rooted with *Macrothylacia rubi* (Lasiocampidae) following recent studies that confidently placed Lasiocampidae as the sister family to remaining bombycoids (43, 51, 52, 58). Further details on the implementation of all phylogenetic methods are found in *Supplementary Information*.

**Divergence Time Estimation.** To estimate divergence times, we used fossil calibrations that met the best practice standards of Parham et al. (59). We also used a calibration on the root node of Sphingidae following the interfamily Lepidoptera dating analysis of Wahlberg et al. (39). We used a Bayesian phylogenetic relaxed molecular-clock model with four calibration points to conduct a divergence time analysis in BEAST 1.7.5 (60). Branch rates were estimated with an uncorrelated relaxed clock model, allowing for the rate of evolution to vary among the branches of the tree with no a priori correlation between the rate of a lineage and that of its ancestor. Parameters were unlinked across partitions, and a Yule tree prior was used, which assumes a constant per lineage selection rate. We used the tree with the highest likelihood from the RAxML analysis and rescaled the tree in r8s 1.8 (61). We used the r8s ultrametric tree as the starting tree for all BEAST runs.

Two fossil-age estimates were based on a lognormal distribution, allowing us to assume that the actual divergence event took place before the earliest appearance of that fossil. For the third fossil age estimate, we used a uniform prior with a rigorous minimum bound because this corresponded to an extant species that was also described as a fossil (*Supporting Information*). A fossil's appearance must postdate the origin of its clade, so it can only provide a minimum age estimate. Because it is unclear by how much the appearance of a clade predates the age of the first fossil, we made sure that the 95% probability included the oldest reasonable age for the clade. We specified the mean, SD, and lower bound for the age of each calibration point. We also ran analyses in which multiple nearby nodes were assigned the same age and subsequently assessed the effect on age estimation, to locate potential sources of error in the estimation process. We outline the reasoning behind our choices for each calibration point in *Supporting Information*.

Angiosperms originated shortly before the ∼125 Ma origin of the eudicots (62–65). Hawkmoth adults are mostly dependent on floral resources from eudicots, and the majority of hawkmoth larvae rely on angiosperms as food plants (19). Therefore, it would be very unlikely for these moths to predate the origin of eudicots or angiosperms. Furthermore, the spilt between Lasiocampidae and remaining Bombycoidea was estimated at ∼94 Ma, and the origin of hawkmoths at ∼75 Ma (39). We therefore assigned the root ingroup to 75 Ma with numerous internal calibration points as described above. We also ran analyses with the age of the root node sampled from a normal and uniform distribution with a mean of 90.0, 85.0, 80.0, 75.0, 70.0, 65.0, and 60.0 ± 1.0 Ma to examine the effect of different ingroup node ages. For these analyses, the start of the Cretaceous was used as an absolute maximum (125 Ma) for the root of the tree, and the youngest age assigned to a fossil was used as the minimum age for that group. We conducted four independent runs of 10 million generations for each of these analyses. Applying a uniform distribution in place of a lognormal distribution to the prior age estimates of the calibration points did not substantially alter our divergence-time estimates. For all dating analyses, we applied a Yule process for model of speciation, and ran the BEAST analysis for 100 million generations. In some cases, when the effective sample size was low for particular parameters, we continued to run the analysis for an additional 50 million generations.

**Ancestral State Reconstruction of Antibat Ultrasound.** Ancestral state reconstruction analyses were conducted to reconstruct the evolutionary history of ears and sound production. We coded the presence and absence of ears based on morphological dissections. Sound production was coded as present or absent for two different categories, based on whether moths made ultrasonic responses to bats and touch. To determine ancestral states of these discrete categories, we ran the analyses in a ML framework in Mesquite (66) and BayesTraits (67), using the RAxML tree (*Supporting Information*). We mapped duty cycle as a continuous character on the ML phylogeny using the contMap function of Phytools v.0.4-07 (68) in R v3.1.0 (69). Duty cycle values were available for 92 taxa in the tree (45.5%), and data for the remaining taxa and their ancestral states were estimated using likelihood by applying the anc.ML function in contMap.

**ACKNOWLEDGMENTS.** We thank J. Breinholt, G. Hill, P. Houlihan, W. Hsu, A. Keener, B. Leavell, C. McClure, and D. Plotkin for extensive assistance; A. Acree, A. Chambers, J. Chave, C. Covell, Jr., F. Dai, J. Daniels, G. Dewsbury, T. Emmel, S. Epstein, M. Espeland, V. Ficarrotta, P. Gaucher, D. Gluckman, A. Hester, N. Homziak, D. Janzen, I. Kitching, G. Lamarre, C. Lopez-Vaamonde, J. Miller, K. Miner, C. Mitter, K. Mitter, Z. Mroz, Y. Paulay, J. Pena, F. Ponce, L. Proux, L. Reeves, J. Regier, C. Romero, R. Rougerie, C. Ryan, C. Segebarth, P. Skelley, S. Sorrell, A. Sourakov, M. Standridge, L. Taylor, J. Troupe, J. Turner, N. Turner, K. Verster, A. Warren, P. Whisler, L. Xiao, J. Yack, M. Zhong, A. Zwick, the many researchers who provided samples, and members of the A.Y.K. and J.R.B. laboratories not listed here; The Danum Valley Field Centre and the Nouragues Research Station for providing outstanding facilities for this research; and the University of Florida High-Performance Computing Cluster for providing necessary computational resources. This project was supported by US National Science Foundation Grants IOS-1121739 and IOS-1121807 (including Research Assistantships for High School Students, Research Experiences for Undergraduates, and Research Opportunity Award supplements) (to A.Y.K. and J.R.B.); US National Science Foundation Grant DBI-1349345 (to A.Y.K.); a Centre National de la Recherche Scientifique Travel grant (to A.Y.K. and J.R.B.); and National Geographic Grant 9107-12 (to A.Y.K.).

1. Teeling EC, et al. (2005) A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* 307(5709):580–584.
2. Simmons NB, Seymour KL, Habersetzer J, Gunnell GF (2008) Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. *Nature* 451(7180):818–821.
3. Conner WE, Corcoran AJ (2012) Sound strategies: The 65-million-year-old battle between bats and insects. *Annu Rev Entomol* 57:21–39.
4. van Nieukerken EJ, et al. (2011) Order Lepidoptera Linnaeus, 1758. *Zootaxa* 3148:212–221.
5. Kristensen NP (2012) Molecular phylogenies, morphological homologies and the evolution of 'moth ears'. *Syst Entomol* 37(2):237–239.
6. Barber JR, Kawahara AY (2013) Hawkmoths produce anti-bat ultrasound. *Biol Lett* 9(4):20130161.
7. Fullard JH, Fenton MB (1977) Acoustic and behavioral analyses of sounds produced by some speceis of nearctic Arctiidae (Lepidoptera). *Can J Zool* 55(8):1213–1224.
8. Ratcliffe JM, Fullard JH (2005) The adaptive function of tiger moth clicks against echolocating bats: An experimental and synthetic approach. *J Exp Biol* 208(Pt 24):4689–4698.
9. Strauß J, Lakes-Harlan R (2014) Evolutionary and phylogenetic origins of tympanal hearing organs in insects. *Insect Hearing and Acoustic Communication*, ed Hedwig B (Springer, Berlin), pp 5–26.
10. Barber JR, et al. (2015) Moth tails divert bat attack: Evolution of acoustic deflection. *Proc Natl Acad Sci USA* 112(9):2812–2816.
11. Bates DL, Fenton MB (1990) Aposematism or startle—Predators learn their responses to the defenses of prey. *Can J Zool* 68(1):49–52.
12. Hristov NI, Conner WE (2005) Sound strategy: Acoustic aposematism in the bat-tiger moth arms race. *Naturwissenschaften* 92(4):164–169.
13. Barber JR, Conner WE (2007) Acoustic mimicry in a predator–prey interaction. *Proc Natl Acad Sci USA* 104(22):9331–9334.
14. Corcoran AJ, Barber JR, Conner WE (2009) Tiger moth jams bat sonar. *Science* 325(5938):325–327.
15. Fullard JH, Fenton MB, Simmons JA (1979) Jamming bat echolocation: The clicks of arctiid moths. *Can J Zool* 57(3):647–649.
16. Fullard JH, Simmons JA, Saillant PA (1994) Jamming bat echolocation: The dogbane tiger moth *Cycnia tenera* times its clicks to the terminal attack calls of the big brown bat *Eptesicus fuscus*. *J Exp Biol* 194:285–298.
17. Corcoran AJ, Barber JR, Hristov NI, Conner WE (2011) How do tiger moths jam bat sonar? *J Exp Biol* 214(Pt 14):2416–2425.
18. Kitching IJ, et al. (2011) CATE Sphingidae. Available at www.cate-sphingidae.org/version May 25, 2011. Accessed April 12, 2014.
19. Kitching IJ, Cadiou JM (2000) *Hawkmoths of the World: An Annotated and Illustrated Revisionary Checklist (Lepidoptera:Sphingidae)* (Cornell Univ Press, Ithaca, NY), p 227.

20. Tuttle J (2007) *The Hawk Moths of North America: A Natural History Study of the Sphingidae of the United States and Canada* (The Wedge Foundation, Washington, DC).
21. Pittaway AR (1993) *The Hawkmoths of the Western Palaearctic* (Harley Books, Colchester, United Kingdom), p 240.
22. Bowers MD (2003) Hostplant suitability and defensive chemistry of the Catalpa sphinx, *Ceratomia catalpae*. *J Chem Ecol* 29(10):2359–2367.
23. Vaughan TA (1976) Nocturnal behavior of the African false vampire bat (*Cardioderma cor*). *J Mammal* 57(2):227–248.
24. Clare EL, Fraser EE, Braid HE, Fenton MB, Hebert PDN (2009) Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): Using a molecular approach to detect arthropod prey. *Mol Ecol* 18(11):2532–2542.
25. Jones G (1990) Prey selection by the greater horseshoe bat (*Rhinolophus ferrumequinum*)—Optimal foraging by echolocation. *J Ecol* 59(2):587–602.
26. Sierro A, Arlettaz R (1997) Barbastelle bats (*Barbastella* spp.) specialize in the predation of moths: Implications for foraging tactics and conservation. *Acta Oecol* 18(2): 91–106.
27. Bohmann K, et al. (2011) Molecular diet analysis of two African free-tailed bats (molossidae) using high throughput sequencing. *PLoS ONE* 6(6):e21441.
28. Pavey CR, Burwell CJ (1998) Bat predation on eared moths: A test of the allotonic frequency hypothesis. *Oikos* 81(1):143–151.
29. Roeder KD, Treat AE, Vandeberg JS (1968) Auditory sense in certain sphingid moths. *Science* 159(3812):331–333.
30. Göpfert MC, Wasserthal LT (1999) Hearing with the mouthparts: Behavioural responses and the structural basis of ultrasound perception in acherontiine hawkmoths. *J Exp Biol* 202(Pt 8):909–918.
31. Roeder KD, Treat AE (1970) An acoustic sense in some hawkmoths (Choerocampinae). *J Insect Physiol* 16(6):1069–1086.
32. Roeder KD, Treat AE, Vande Berg JS (1970) Distal lobe of the pilifer: An ultrasonic receptor in choerocampine hawkmoths. *Science* 170(3962):1098–1099.
33. Clare EL, Symondson WO, Fenton MB (2014) An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). *Mol Ecol* 23(15):3633–3647.
34. Kick SA, Simmons JA (1984) Automatic gain control in the bat's sonar receiver and the neuroethology of echolocation. *J Neurosci* 4(11):2725–2737.
35. Barber JR, Conner WE (2006) Tiger moth responses to a simulated bat attack: Timing and duty cycle. *J Exp Biol* 209(Pt 14):2637–2650.
36. Corcoran AJ, Conner WE, Barber JR (2010) Anti-bat tiger moth sounds: Form and function. *Curr Zool* 56(3):358–369.
37. Kawahara AY, Mignault AA, Regier JC, Kitching IJ, Mitter C (2009) Phylogeny and biogeography of hawkmoths (Lepidoptera: Sphingidae): Evidence from five nuclear genes. *PLoS ONE* 4(5):e5719.
38. Heffner R, Heffner H (2010) Explaining high-frequency hearing. *Anat Rec (Hoboken)* 293(12):2080–2082.
39. Wahlberg N, Wheat CW, Peña C (2013) Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). *PLoS ONE* 8(11):e80875.
40. Hristov N, Conner WE (2005) Effectiveness of tiger moth (Lepidoptera, Arctiidae) chemical defenses against an insectivorous bat (*Eptesicus fuscus*). *Chemoecology* 15(2):105–113.
41. Wink M, Theile V (2002) Alkaloid tolerance in *Manduca sexta* and phylogenetically related sphingids (Lepidoptera: Sphingidae). *Chemoecology* 12(1):29–46.
42. Maclean DB, Sargent TD, Maclean BK (1989) Discriminant-analysis of lepidopteran prey characteristics and their effects on the outcome of bird-feeding trials. *Biol J Linn Soc Lond* 36(3):295–311.
43. Regier JC, et al. (2013) A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). *PLoS ONE* 8(3):e58568.
44. Cho S, et al. (2011) Can deliberately incomplete gene sample augmentation improve a phylogeny estimate for the advanced moths and butterflies (Hexapoda: Lepidoptera)? *Syst Biol* 60(6):782–796.
45. Mutanen M, Wahlberg N, Kaila L (2010) Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. *Proc Biol Sci* 277(1695): 2839–2848.
46. Timmermans MJTN, Lees DC, Simonsen TJ (2014) Towards a mitogenomic phylogeny of Lepidoptera. *Mol Phylogenet Evol* 79:169–178.
47. Masters WM (1979) Insect disturbance stridulation: Its defensive role. *Behav Ecol Sociobiol* 5(2):187–200.
48. Conner WE (1999) 'Un chant d'appel amoureux': Acoustic communication in moths. *J Exp Biol* 202(Pt 13):1711–1723.
49. Mell R (1922) *Biologie und Systematik der Südchinesischen Sphingiden* (R. Friedländer & Sohn, Berlin), p 331.
50. Tinbergen N (1963) On aims and methods of ethology. *Z Tierpsychol* 20(4):410–433.
51. Zwick A, Regier JC, Mitter C, Cummings MP (2011) Increased gene sampling yields robust support for higher-level clades within Bombycoidea (Lepidoptera). *Syst Entomol* 36(1):31–43.
52. Regier JC, et al. (2008) Phylogenetic relationships of wild silkmoths (Lepidoptera: Saturniidae) inferred from four protein-coding nuclear genes. *Syst Entomol* 33(2): 219–228.
53. Regier JC, Cook CP, Mitter C, Hussey A (2008) A phylogenetic study of the 'bombycoid complex' (Lepidoptera) using five protein-coding nuclear genes, with comments on the problem of macrolepidopteran phylogeny. *Syst Entomol* 33(1):175–189.
54. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* 30(4):772–780.
55. Lanfear R, Calcott B, Ho SYW, Guindon S (2012) Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol* 29(6):1695–1701.
56. Breinholt JW, Kawahara AY (2013) Phylotranscriptomics: Saturated third codon positions radically influence the estimation of trees based on next-gen data. *Genome Biol Evol* 5(11):2082–2092.
57. Ronquist F, et al. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61(3):539–542.
58. Zwick A (2008) Molecular phylogeny of Anthelidae and other bombycoid taxa (Lepidoptera: Bombycoidea). *Syst Entomol* 33(1):190–209.
59. Parham JF, et al. (2012) Best practices for justifying fossil calibrations. *Syst Biol* 61(2): 346–359.
60. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7(1):214.
61. Sanderson MJ (2003) r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19(2):301–302.
62. Doyle JA (2012) Molecular and fossil evidence on the origin of angiosperms. *Annu Rev Earth Planet Sci* 40:301–326.
63. Bell CD, Soltis DE, Soltis PS (2010) The age and diversification of the angiosperms re-revisited. *Am J Bot* 97(8):1296–1303.
64. Jiao Y, et al. (2011) Ancestral polyploidy in seed plants and angiosperms. *Nature* 473(7345):97–100.
65. Magallón S, Hilu KW, Quandt D (2013) Land plant evolutionary timeline: Gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. *Am J Bot* 100(3):556–573.
66. Maddison WP, Maddison DR (2014) Mesquite: A modular system for evolutionary analysis. Version 3.01. Available at mesquiteproject.org. Accessed August 17, 2014.
67. Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am Nat* 167(6):808–825.
68. Revell LJ (2012) Phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3(2):217–223.
69. R_Development_Core_Team (2014) *R: A Language and Environment for Statistical Computing*. (R Foundation for Statistical Computing, Vienna). Available at www.R-project.org. Accessed August 18, 2014.