Adaptations to divert the attacks of visually guided predators have evolved repeatedly in animals. Using high-speed infrared videography, we show that luna moths (Actias luna) generate an acoustic diversion with spinning hindwing tails to deflect echolocating bat attacks away from their body and toward these nonessential appendages. We pit luna moths against big brown bats (Eptesicus fuscus) and demonstrate a survival advantage of ~47% for moths with tails versus those that had their tails removed. The benefit of hindwing tails is equivalent to the advantage conferred to moths by bat-detecting ears. Moth tails lured bat attacks to these wing regions during 55% of interactions between bats and intact luna moths. We analyzed flight kinematics of moths with and without hindwing tails and suggest that tails have a minimal role in flight performance. Using a robust phylogeny, we find that long spatulate tails have independently evolved four times in saturniid moths, further supporting the selective advantage of this anti-bat strategy. Diversionary tactics are perhaps more common than appreciated in predator–prey interactions. Our finding suggests that focusing on the sensory ecologies of key predators will reveal such countermeasures in prey.

Significance

Bats and moths have been engaged in acoustic warfare for more than 60 million y. Yet almost half of moth species lack bat-detecting ears and still face intense bat predation. We hypothesized that the long tails of one group of seemingly defenseless moths, saturniids, are an anti-bat strategy designed to divert bat attacks. Using high-speed infrared videography, we show that the spinning hindwing tails of luna moths lure echolocating bat attacks to these nonessential appendages in over half of bat–moth interactions. Further we show that long hindwing tails have independently evolved multiple times in saturniid moths. This finding expands our knowledge of antipredator deflection strategies, the limitations of bat sonar, and the extent of a long-standing evolutionary arms race.

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strikes. We did so by reviewing three different high-speed camera angles of the interactions. Bats aimed at tails in 55.2% (n = 87) of interactions with intact moths (Movie S2), slightly more often than they aimed at the moth’s body (44.8%, n = 87; binomial test, P = 0.05; Movie S3). Visual deflection marks are also usually found on the posterior of prey animals, which likely draws the predators’ attack away from the escape trajectory (1–7). In fact, bats were rarely successful at capturing moths when they aimed at tails (4.2%, n = 48) but showed similar capture success to moths with tails removed when they targeted the body (71.8%, n = 39; binomial test, P = 0.07).

Bats often use an aerobatic capture technique that involves guiding the prey with a wing toward the midline of the bat before bringing the tail membrane toward the head where the insect can be bitten (Movie S4). In a more direct strategy, bats surround the prey with the wings and tail membrane concurrently and, in one motion, bring the insect to the mouth (Movie S3). When the bats in these experiments hunted intact luna moths, they generally approached from the side or behind the flying moth, using a direct strategy more often, biting and removing a tail (6.9%; Movie S5), damaging a tail (8.1%), or removing a section of hindwing near the tail (5.7%) in 20.7% (n = 87) of interactions. Once a moth was subdued, the bats in these experiments targeted the thorax first, likely to damage the moth’s motor center and prevent it from escaping. Our data indicate that moth tails redirect these lethal attacks to expendable wing areas.

An alternative explanation of our results is that bats simply had a harder time capturing intact luna moths due to their greater wing size (11). To examine this possibility, we pit Antheraea polyphemus saturniid moths against the same bats, which are larger in area (body + wing, 71.6 cm²) than intact luna moths (48.1 cm²) yet lack tails. We found evidence that size does indeed provide a dividend: 66% (n = 50) of these moths were captured. However, this survival benefit compared with ablated luna moths does not explain the greater capture success of A. polyphemus compared with intact luna moths (Fig. 1; mixed-effects logistic regression, P < 0.05). Clearly, tails provide an anti-bat advantage beyond increased size alone. Enlarged and lobed hindwings might be functional intermediates on the way to the evolution of tails.

We cannot differentiate whether bats are targeting moth tails because they are a conspicuous element of a single target or are perceived as an alternative target. We might expect that information from multiple targets would increase attack latency (3), however using mixed-effects linear regression, we found no difference in latency between intact moths and those without tails (t = −0.52). Preliminary ensonification experiments indicate tails create distinct wing-like amplitude and frequency modulations on the returning echo stream of synthetic frequency-modulated (FM) signals (Fig. S2). Bats that use constant-frequency echolocation use the small amplitude and frequency modulations imposed on the returning echoes from beating insect wings to localize and discriminate prey (12, 13). However, only limited evidence indicates bats that emit FM sonar might do so, using information either within a single echo (14) or across an echo strobe group (15, 16). Regardless, the FM bats in these experiments were lured to the echoes of spinning tails.

To understand the historical pattern of tail evolution, we measured tail lengths of 113 saturniid moth species and constructed a phylogeny using maximum likelihood (ML) and Bayesian approaches. Trees from both analyses provided very similar results that are largely congruent (Fig. 2 and Fig. S3). Our results demonstrate four independent origins of long (>30 mm) hindwing tails with modified spathulate tips in the Saturniidae. Tails evolved in the well-supported Actias + Argema + Glaresia clade, Copiopteryx, Eudamonia, and Coscinocera, which are placed in disparate tribes and two different saturniid subfamilies. Evidence for a single origin of tails is strongly rejected statistically (SH test, P < 0.0001). Examination of the tail moth clades appears to show tail length increases from taillless or ancestrally shorter tails. Tail length might be increasing under selection from bats to move the echoic target created by spinning tails further from the body and forewings. Although a phylogenetic study with increased sampling focusing on these tailed clades is needed to support this conclusion, we note forewing damage in 8% (n = 87) of bat–luna moth interactions. It would be informative to look broadly across the Saturniidae and assess both the rates of damage to critical flight infrastructure (17) and bat capture rates of moths with longer (e.g., Copiopteryx, >100 mm) and shorter (e.g., Asenura, <10 mm) tails than luna moths (average 37.5 mm).

Our data clearly support the anti-bat function of hindwing tails in luna moths, yet other selective forces might also be at work. Some saturniid moths are sexually dimorphic in wing morphology, including longer tails in males (18). However, it is difficult to separate sexual selection from the markedly different life history pressures on the sexes. Females often do not fly until mated and spend most of their short adult lifespan (average 4–8 d) disseminating pheromone from protected sites, dispersing short distances to oviposit (11). Male moths are the primary sex searching for mates and are under greater threat of bat predation (19). Saturniid moths are not known to use visual cues during mating, and the majority of species, including those with tails, are monandrous, with females tending to mate with the first available male (20). Thus, it is unlikely that tails are used in mate choice. We view the possible influence of sexual selection on tails to be in addition to, not instead of, natural selection from bat predation.

Moth tails might also function in flight performance. To begin examining this possibility, we filmed luna moths flying in our flight room with (n = 12) and without (n = 14) hindwing tails with three synchronized, high-speed cameras. After handling the moths, we released them from one corner of the flight room and reconstructed their 3D escape flights. We calculated the mean values for wingbeat frequencies, speed, acceleration, normal acceleration (rate of direction change), normal acceleration in the horizontal plane (rate of direction change in the horizontal plane), and curvature (a measure of flight erraticism) over the flight period (Table S1). Due to highly correlated flight parameters (r > 0.5), we limited our analyses to speed, curvature, and wingbeat frequency. Logistic regression revealed that wingbeat frequency was the only parameter that significantly changed when hindwing tails were ablated (P = 0.03). The increase in wingbeat from 10 to 11 Hz in ablated moths likely indicates the moths were compensating for reduced lift or were less constrained by the reduced drag or mass from the removed hindwing area, but average kinematics involved in normal flight and predator evasion did not change.

More than half of the ∼140,000 nocturnal moth species possess ears specialized to detect bat sonar (21). However, >65,000 species of nocturnal moths lack this acoustic defense (22) yet still face intense bat predation (23). Our data suggest that diversification anti-bat defenses can be as successful as other acoustic strategies.
in this arms race (24–26). Additional taxa with hindwing tails, including Lepidoptera and lacewings (27), might also be diverting predator attack to nonessential appendages. Our findings suggest that focusing on the sensory ecologies of important predators will reveal additional diversionary tactics in animals.

Materials and Methods

Animals. We mistnetted eight adult female big brown bats (E. fuscus) in Idaho and maintained them on a diet of mealworms (Tenebrio larvae) and moths used in experiments along with vitamin supplements. Recent molecular diet analyses reveal that E. fuscus consistently preys on Lepidoptera (28), making it a suitable model for these investigations. Actias luna and A. polyphemus moths were purchased as pupae from local providers, as pupae, larvae, or eggs, or collected as adults (females only) in Gainesville, Florida, and placed into glassine envelopes where eggs were laid. We reared larvae in a temperature-controlled indoor laboratory at the Florida Museum of Natural History, McGuire Centre for Lepidoptera and Biodiversity (MGCL), in glass terrariums (30” length × 12” width × 12” height) and fed them sweet gum (Liquidambar styraciflua) as their primary host plant. We used pyralid moths (Galleria mellonella), purchased as pupae from local providers, as positive controls in experiments.
Behavioral Experiments and Statistical Analysis. We conducted all vertebrate work with approval from Boise State University’s Animal Care and Use Committee (IACUC 006-02015) in a foam-lined indoor flight facility (7.6 m × 6.7 m × 3 m). We tethered moths to an 85-cm monofilament line through a small hole in the prothorax and visually compared the flight behavior of several moths tethered with this method to another approach that involved supergluing the line to the moth’s prothorax. Because there were no differences in flight behavior, we used the former method to prevent the bats from ingesting glue. Eight bats hunted saturniid and control pyralid moths for 1–7 nights (average 3.75) each. We presented an approximate 70:30 ratio of male/female saturniid moths to bats; the species we used in these experiments are not sexually dimorphic in shape, but females are slightly larger in both species (18). To record each bat–moth interaction, we used three digital, high-speed, infrared-sensitive video cameras (Basler Scout, 120 frames per second) streaming to a desktop computer via a National Instruments PCI-e8235 Giga Vision frame grabber and custom LabView software. We illuminated the interaction space with eight infrared Wildlife Engineering LED arrays. To record echolocation, we mounted four ultrasonic condenser microphones [Avisoft CM16, −3 dB b, 20–140 kHz] on the ceiling, in the four cardinal directions, 85 cm from the attachment point of the tether. XLR cables connected the microphones to a four-channel Avisoft UltraSoundGate 416H (sampling at 250 kHz) recording to a desktop computer. We used the pulsed-sonar echolocation software and audio by triggering both with a National Instruments 9402 digital I/O module. We performed all statistical tests in R (29) and conducted mixed-effects regressions using the package lme4 (30). All mixed-effects regressions included the individual bats as random effects.

Echolocation Analysis. Using Avisoft SASLab Pro software, we analyzed the echolocation calls to estimate detection distances for each side of the flight for species of luna moths and for species of A. polyphemus. We recorded videos of A. luna for video DDC sonar and the microphones as R1 vein meets the base of the wing to the wing apex) with a digital Instruments 9402 digital I/O module that triggered both the video and audio. We ensonified luna moths under three conditions: (i) normal eclipse, the component of acceleration due to the change of direction of the moth’s flight path, and (ii) the horizontal normal acceleration, the component that occurs within the horizontal plane. To standardize the analyses of flights that varied in their duration, we calculated the mean values for all five variables during a 1-s flight period, beginning 20–50 frames after the moth was released to minimize the effects of the initial release on moth flight kinematics.

Calculating Average Body and Wing Area Size. We photographed six specimens of A. luna and A. polyphemus from the MGGL collections. To a known distance on a white background, we attached specimens to a 3-mm opaque acrylic sheet and illuminated the area with two Yongnuo flashguns. We placed a reference scale near the specimens and photographed each individual at a consistent distance. We imported JPG files into ImageJ for analysis. A scale was set at 183.33 pixels/cm, and all images were changed to 8-bit. We measured the overlapping area of the forewing and hindwing, measured the head and antennae, and subtracted that measurement from the total wing and body area size.

Saturniid Tail Measurements. We obtained specimens for tail measurements from the dried pinned collection of the MGGL. For each species, we measured up to 30 specimens that were available. We measured the width of the tail (from the base of the tail to the wing base), and we chose the smallest tail that each specimen had. We measured the tail width with a digital caliper to correct for size. When there was damage to the right wings, we chose the equivalent wing from the left pair. All wing measurements are included in Dataset S1.

Phylogenetic Methods. Using available nucleotide data from GenBank (35) and BOLD, we assembled data for five nuclear loci (CAD, DDC, EF-1, Period, and Wingless) and the COI mitochondrial gene for 80 taxa from Saturniidae and 34 taxa representing closely related bombycid families, based on relationships inferred from recent deep phylogenetic studies of Bombycoidea (36–39). In individual loci were aligned with MAFFT v7.130b (40) and concatenated using Geneious 5.5.6 (www.geneious.com) and converted to the nexus format. We used PartitionFinder v1.1.1 (41) on the concatenated alignment to search for the best partitioning strategy and models based on genes and codon positions using a greedy algorithm and the Bayesian information criterion (BIC) score (Dataset S1). Using RAxML 8.0.24 (42), we estimated phylogenetic trees with ML and ran Bayesian analyses with MrBayes 3.2 (43). We partitioned the dataset in RAxML following PartitionFinder results and applied the GTR–ΓA-MMA model to all partitions and searched for the best tree with the −d option using 200 random starting topologies and a combined bootstrap and likelihood search for 1,000 bootstrap pseudoreplicates with the −f a option following our previously published approach (44). For the Bayesian analysis, we followed the same partition scheme as used in RAxML but used PartitionFinder to optimize the models available in MrBayes. In MrBayes, we ran four independent Markov Chain Monte Carlo chains that consisted of one cold chain and three hot chains. Each run used default flat priors and was started from a random tree for 3 × 107 generations sampling every 1,000 generations. The variables
statefreq, revmat, shape, and pinvar were unlinked across all partitions, and models of evolution were set following PartitionFinder BIC results. To determine burn-in length, we made sure the average SD of split frequencies between runs fell below 0.01 and further used the program Tracer v1.5 (45) to check for convergence of the negative log likelihood values between chains. After deletion of burn-in, we combined MrBayes runs to make a majority-rule consensus tree with posterior probabilities.

### Ancstral State Analysis and Statistical Tests of Tail Origins

We used the ML tree estimated in RaxML to conduct all ancstral state analyses. We first examined the number of origins of tails in Saturniidae with Mesquite 3.01 (46) using the continuous character mapping “contMap” in Phytools version 0.2–20 (48) that implemented the “fastAnc” function that computes ancstral states with Felsenstein contrasts algorithm (49) for all internal nodes (Fig. S5).

To correct for body size on phylogeny, we calculated the average ratio of tail length to body size (Supporting Information) and mapped these values on the RaxML tree with the contMap function in Phytools (Fig. S6). To test whether multiple origins of hindwing tails were more likely than a single origin, we conducted an SH test (50) on the ML topology in RaxML.

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