

Research



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Predation risks of signalling and searching: bats prefer moving katydids

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Males signalling their attractiveness to females are at risk from predators that exploit mating signals to detect and locate prey. Signalling, however, is not the only risky activity in sexual interactions: mate searching can incur risk as well. Male Neotropical pseudophylline katydids produce both acoustic and vibrational signals (tremulations). Females reply to male signals with tremulations of their own, and both sexes walk to find one another. We asked if movement increases predation risk, and whether tremulation or walking was more attractive to predators. We offered the Neotropical gleaner bat *Micronycteris microtis* a series of two-choice tests, presenting the bats with katydid models that were motionless or moved in a way to mimic either tremulating or walking. We found that prey movements do put prey at risk. Although *M. microtis* can detect motionless prey on leaves, they preferred moving prey. Our study shows that movement can put searching or signalling prey in danger, potentially explaining why silent female katydids are frequently consumed by gleaner bats.

1. Introduction

Reproducing can be dangerous for animals. The process of finding a mate, and mating itself, can increase the risk of predation by generating cues for predators [1–3] or by reducing the responsiveness of animals to predator cues [4–7]. Research on this topic has largely focused on the risk to males that signal to attract females and predators that eavesdrop on these male signals [8–11]. Predators, however, are also highly attracted to movement [12–14], so females can experience high predation risk as they move to locate signalling males [15–19]. The risks of signalling and searching are often measured separately, even though studies on multiple signalling individuals demonstrate that the risk of predation is not just related to the animal's own behaviour, but behaviour of neighbouring animals as well ('collateral damage': [20,21]). To understand the relative predation risks faced by signallers and searchers, it is, therefore, important to test predator responses not only to prey signals but also to nearby prey movements towards the signal, reflecting how these interactions occur in nature.

In most katydid and cricket species, males produce acoustic signals and females find males by phonotaxis, i.e. tracking the source of the sound [22]. Neotropical pseudophylline katydids, however, have a duetting communication system in which males also produce separate vibrational signals, called tremulations, and females reply to male tremulations with tremulations of their own [23–25]. Tremulations can be detected by other individuals located on the same substrate, and both males and females walk to find the other. Therefore, once in close proximity on a plant, each individual can influence the relative predation risk of the other—either by being motionless, signalling, or by walking to the signaller. This mating system provides an opportunity to test how the behaviour of one individual influences the predation risk of the other in a mate-finding context.

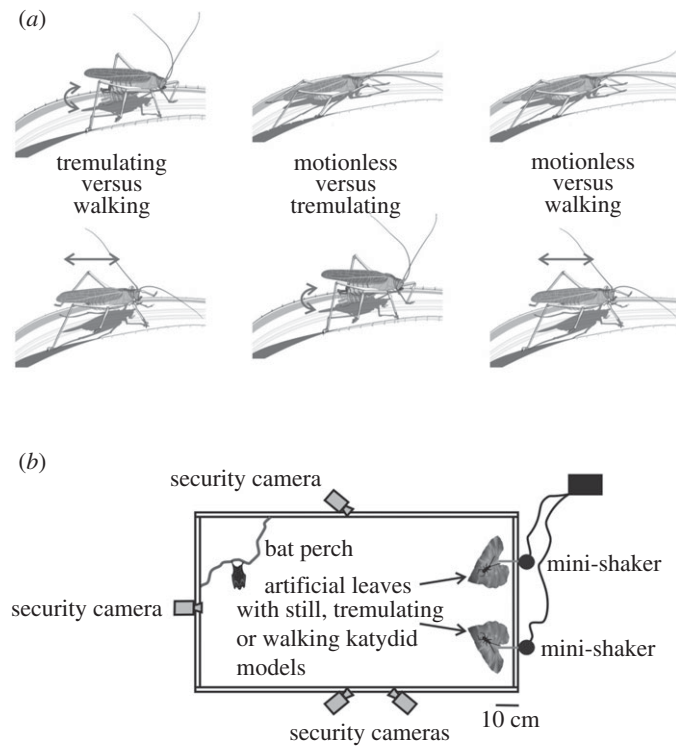


Figure 1. (a) Choice tests: tremulating versus walking katydid; motionless versus tremulating katydid; motionless versus walking katydid. Arrows indicate the direction of movement. (b) Experimental set-up: flight cage viewed from above with the two experimental leaves on the right wall. The movement of a model katydid on each experimental leaf was controlled by a minishaker (tremulation) or fishing line (walking). Katydid illustrations by Damond Kylo.

Neotropical pseudophylline katydids experience intense predation pressure from bats that glean prey from vegetation [23,25,26]. Many gleaning bats eavesdrop on the acoustic signals of male katydids to locate them as food [27–29]. It is hypothesized that the intense predation pressure by gleaning bats selected for both the severely reduced acoustic signalling observed in Neotropical pseudophylline katydids and the evolution of vibrational signalling as a communication channel that is hidden from acoustically eavesdropping bats [23,24,28,30]. However, diet studies for Neotropical gleaning bat species show that at least half of the pseudophylline katydid prey are silent females [26,28], suggesting that movement might also be an important cue used by bats. The goals of this study are to (i) determine if Neotropical gleaning bats use movement as a cue to detect insect prey and (ii) assess the relative risk of either being motionless, signalling through tremulation, or walking when courting pseudophylline katydids interact.

We conducted choice experiments with the Neotropical gleaning bat species *Micronycteris microtis* to test the hypothesis that movement is a salient cue for locating prey in some gleaning bats. We selected *M. microtis* because it eats a significant number of katydids ([31], I.G. 2016 to present, unpublished data) and uses echolocation to detect silent, motionless prey [32,33]. For this species' prey, staying still is not necessarily an effective antipredator strategy—but it might lower risk relative to individuals moving nearby.

2. Material and methods

(a) Study animals

We captured 10 experimentally naive adult *M. microtis* (seven females, three males) from Soberanía National Park, Panamá (N 09°04.640'; W 079°39.077'). Bats were initially released as a group into a small indoor flight cage (1.4 × 1.0 × 0.8 m) containing

four plastic-coated fabric leaves on one wall, each with one dead katydid attached to it. Once all individuals had successfully gleaned a prey item off a leaf, each bat was tested individually with experimental stimuli in the same flight cage.

(b) Experimental set-up and stimuli

To test the relative predation risk of insect movements in the mating context, we presented bats with soft plastic model katydids (GN100, Dixie Novelty Distributors, Bucyrus, OH, USA) that were either motionless on an artificial leaf (no movement), shaking perpendicular to the leaf surface (to simulate katydid tremulations), or moving up and down the leaf (to simulate walking). We measured the displacement, which is the largest distance moved by the katydid head during the walking or tremulating simulation, using a ruler (Helix®, Maped Helix, Kingswinford, West Midlands, UK). The tremulating katydid moved away and towards the leaf by less than 1 cm and the walking katydid moved up and down the leaf by about 15 cm, a displacement difference of an order of magnitude. As the bat is continuously changing its position relative to the leaf surface [33], this causes a change in angle and direction of the movement of the prey for the bat, with the axes of the prey's movement changing relative to the bat as the bat moves.

Each bat was presented with three two-choice tests in random order: (i) motionless versus tremulating katydid, (ii) motionless versus walking katydid, and (iii) tremulating versus walking katydid (figure 1a). The two treatments for each test were presented on the upper two artificial leaves (figure 1b) and treatment side (left or right leaf) was alternated for each bat. Model soft plastic katydids were used to eliminate reliance on potential odour cues. Bats were tested under infrared light. We recorded the overall bat behaviour with four infrared-sensitive cameras (12fps; Circuit CR-AHD8 B80-A, Circuit City, Panama City, Panama).

The motionless treatment was the katydid model mounted on a wooden dowel such that the katydid was positioned just above the leaf. To simulate tremulations, we recorded three *Docidocercus gigliotosi* (Griffini 1896; Pseudophyllinae) katydids tremulating on

a leaf using a laser Doppler vibrometer (Polytec PDV100 with Vib-E-220 DAC, Polytec, Waldbronn, Germany). The laser was pointed at the dorsal midpoint of the abdomen perpendicular to the main axis of the katydid's body. Tremulation signals were recorded using Avisoft Lite recording software (v. 5.2.12, Avisoft Bioacoustics, Glienicke, Germany). The recorded signals were played back using Avisoft Lite (v. 5.2.12, Avisoft Bioacoustics) via an amplifier (Dayton Audio DTA-120, Springboro, OH, USA) connected to a minishaker (Model 4810, Brüel & Kjær, Nærum, Denmark) moving the dowel with the mounted model. The dowel was connected to the shaker positioned behind the leaf, allowing for the movement of the katydid above the leaf surface but preventing movement of the leaf itself. This set-up was intended to simulate typical katydid tremulations: large movements of the abdomen with little movement of the leaf. We calibrated the tremulation amplitude of the shaking model by measuring with the laser vibrometer and matching its average velocity amplitude to that of the live katydids at the peak tremulation frequency. Tremulation signals of each of the three recorded katydids were assigned randomly to the bats. For the walking treatment, we used fishing line to move the katydid model up and down the leaf just above the leaf surface to avoid producing sounds. The line was moved at a steady rate by the same person in all trials, who was unaware of the bats' choices. The displacement of the model (from bottom to top of the leaf) was standardized by markers on the line by which the controller was able to judge when to switch the movement direction. The movement rate (approx. 3 cm s^{-1}) was estimated from observations of katydids walking on leaves during mate searching. We acknowledge that these treatments are not perfect representations of the natural movements, but they are intended to represent different categories of movements exhibited during katydid courtship behaviour.

(c) Scoring and analysis

For each test, we simultaneously presented two treatments to each bat until it left the perch, flew towards one of the two katydid models and started a short stereotypic flight behaviour in front of it, previously defined as scanning behaviour (for details, see [32]). We scored the bat's choice as the first stimulus it approached and scanned. After completing the experiments, we confirmed each choice and measured the duration of each scanning bout using Solomon Coder (v. 17.03.22, <https://solomoncoder.com>).

(d) Statistical analysis

We used binomial tests to assess if the bats made non-random choices within each choice test. To test whether the bats preferred the tremulating or walking katydid over a motionless one across all trials, we used a non-parametric permutation test to compare the observed frequency of bats choosing a moving katydid with the expected frequency under the null hypothesis. To create a distribution of expected values under the null hypothesis, we generated 5000 outcomes of bats choosing randomly. To estimate two-sided p -values, we doubled the proportion of expected values that were greater than the observed value. To test whether bats spent more time scanning the tremulating or walking katydid models compared to the motionless model, we compared the scanning durations within each bat using permuted paired t -tests. We used non-parametric bootstrapping to calculate the 95% confidence interval for the mean difference in time spent at each katydid type. All statistical tests were performed in R (v. 3.4.0, <https://www.r-project.org/>).

3. Results

Typically, the bats would scan one of the presented stimuli and then move on. We observed one attack, which occurred

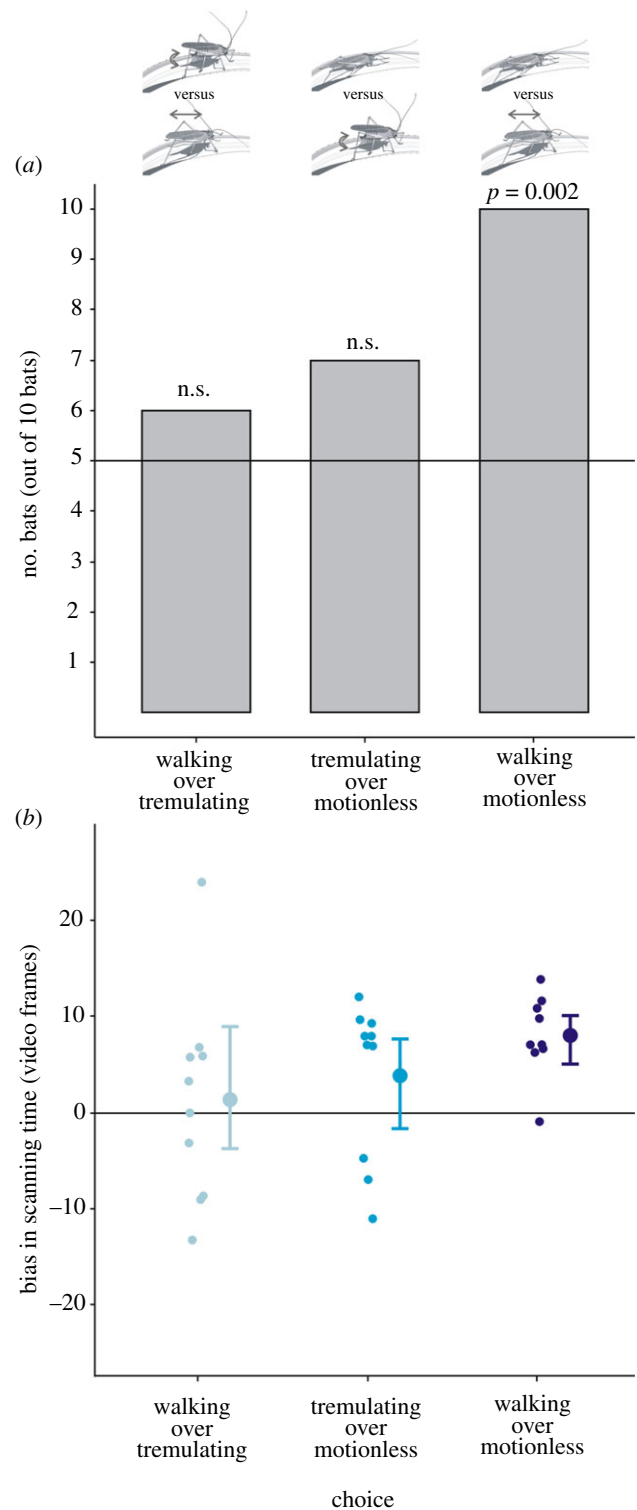


Figure 2. Bats prefer a moving katydid: (a) number of bats ($n = 10$) that first chose the katydid that was walking over tremulating, tremulating over motionless or walking over motionless. (b) Mean difference (and bootstrapped 95% CI) in the scanning duration (number of frames) for each choice.

on a tremulating katydid in a choice between the tremulating and still treatment.

In general, bats preferred the katydid with greater displacement (permutation tests on choices: $p = 0.004$). All 10 bats chose the walking over the motionless katydid (binomial test, $p = 0.002$; figure 2a). Seven of 10 bats chose the tremulating katydid over the motionless katydid (binomial test, $p = 0.34$; figure 2a). Six of 10 bats chose the walking katydid over the tremulating katydid (binomial test, $p = 0.75$; figure 2a). The bats

also spent more time scanning the katydid models that were moving (figure 2c; permutation test with scanning times, $p = 0.002$). These results, taken together, are consistent with the hypothesis that bats preferred a moving target, and possibly those with greater displacement.

4. Discussion

Our study demonstrates that the gleaning bat *M. microtis* can use movement as a cue for locating prey and that movement does indeed put prey at greater risk of detection by a predator. *Micronycteris microtis* preferred walking katydid models to still ones in every trial, and overall, they more often chose moving prey with greater amplitude movements than prey with no movement or lower amplitude movements. Bats also scanned moving prey for longer periods of time. A previous study shows that *M. microtis* can discriminate between different prey items [32], including artificial dummies, which might explain the reluctance of most bats to land on or attack our models. We are confident, however, that the bats initially detected the models as potential prey because they flew towards and scanned them, behaviour that does not occur without a prey target, and also because one bat attacked the model.

Micronycteris microtis is one of the few gleaning bat species known so far to use echolocation for the detection of silent and motionless prey [33]. Many Neotropical gleaning bat species, however, eavesdrop on the acoustic signals of male katydids to locate them [27–29]. Neotropical katydids have very low calling rates, presumably as a defence against gleaning bats [28,34]. It has been suggested that vibrational signalling in some species is a communication channel potentially hidden from these bats [28]. Future research should test if eavesdropping bats that are passive listeners of the acoustic signals of katydid can also use insect movements for prey detection, and to what extent, echolocating bats can discriminate subtle prey movement. If detection of subtle movement cues is common across Neotropical gleaning bats, then tremulations might pose an additional risk for signalling katydids.

It is frequently assumed that signalling males bear the greater risk of predation than females due to the conspicuousness of their sexual advertisement signals [1]. However, this study and previous work [19] suggest that female katydids and crickets might experience similar predation risk to males, due to cues generated through moving towards male signals when searching for mates. Indeed, in the closely related and sympatric bat species, *Micronycteris hirsuta*, approximately

half of the consumed katydids are female [26,28]. However, it is currently unknown whether *M. hirsuta* relies more on passive listening or active echolocation to detect prey.

Finally, our results suggest that the actions of neighbouring individual prey can affect the risk incurred by any single individual—particularly if actions differ in their degree of conspicuousness, as is often the case in mating duets. In Neotropical pseudophylline katydids, both sexes may walk, tremulate and remain still over the course of a mate-finding or courtship duet. Our results indicate that either vibrational signals or the movement towards a signaller might increase predation risk.

Here, we demonstrate that the gleaning bat *M. microtis* pays attention to movement cues to locate prey. In many movement-based courtship interactions, both partners are likely at higher predation risk, with their relative risk changing throughout the interaction. Our results provide insight into the complexity of the interactions between hunting bats and their katydid prey including the potential effect of the searcher on the signaller, and suggest possible new avenues for future research assessing selection pressure on prey-finding strategies in gleaning bats and predator avoidance in Neotropical katydids.

Ethics. Our experiments adhere to ABS/ASAB guidelines for ethical treatment of animals and were approved by the Government of Panamá (Ministerio de Ambiente permit nos. SE/A-25-18; SE/AP-13-18) and the Smithsonian Tropical Research Institute (STRI IACUC protocols 2016-0101-2019; 2019-0102-2022, 2017-0102-2020).

Data accessibility. The data and R script to reproduce these analyses are available as electronic supplementary material and available from the Dryad Digital Repository: <https://dx.doi.org/10.5061/dryad.sf7m0cg2c> [35].

Authors' contributions. I.G. conceived the original idea. I.G., C.E.K., R.A.P. and H.M.t.H. conceived and designed the study. I.G., C.E.K. and A.S.L. designed the behavioural experiment. C.E.K. and A.S.L. collected and analysed the behavioural data. G.G.C. carried out the statistical analyses. I.G., R.A.P., C.E.K. and H.M.t.H. wrote the manuscript. All authors revised the manuscript critically and gave final approval for publication. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. We declare we have no competing interests.

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