We used both in vivo and in vitro activity assays to investigate the mechanism of Sep-tRNA to Sec-tRNA conversion by human SepSecS. First, the reduction of the Schiff base by sodium borohydride to form a chemically stable secondary amine and thus to cross-link PLP to Lys^{284} renders SepSecS completely inactive in vitro (fig. S4B). The catalytic activity of SepSecS is also quenched on removal of PLP by treatment with hydroxylamine (fig. S4B). Some residual activity that is observed after hydroxylamine treatment is probably because of incomplete removal of PLP (fig. S4B). Second, we show that the Arg75Ala, Gln105Ala, and Arg313Ala mutants are inactive in vivo (fig. S4A). These residues are involved in coordinating either the phosphate group of PLP or that of Sep. Finally, the in vivo activities of the Arg97Ala, Arg97Gln, Lys173Ala, and Lys173Met mutants are indistinguishable from that of the wild-type enzyme, which confirms that Arg^{97} and Lys^{173} are involved only in the nonproductive binding of free Sep (fig. S4A).

On the basis of our findings, we propose the following PLP-based mechanism of Sep-tRNA to Sec-tRNA conversion. The reaction begins by the covalently attached Sep being brought into the proximity of the Schiff base when Sep-tRNA^{Sec} binds to SepSecS. The amino group of Sep can then attack the Schiff base formed between Lys^{284} and PLP, which yields an external aldimine (Fig. 4, A and B). The reoriented side chain of Lys^{284} abstracts the Cα proton from Sep (Fig. 4C), and the electron delocalization by the pyridine ring assists in rapid β-elimination of the phosphate group, which produces an intermediate dehydroalanyl-tRNA^{Sec} (Fig. 4, C and D). After phosphate dissociation and binding of selenophosphate, the concomitant attack of water on the selenophosphate group and of the nucleophilic selenium onto the highly reactive dehydroalanyl moiety yield an oxidized form of Sec-tRNA^{Sec} (Fig. 4D). The protonated Lys^{284} returns the proton to the Cα carbon and then attacks PLP to form an internal aldimine (Fig. 4E). Finally, Sec-tRNA^{Sec} is released from the active site (Fig. 4F).

This mechanism is clearly distinct from the persulfide-intermediate mechanism in the Sep-tRNA^{Cys} to Cys-tRNA^{Cys} reaction (22) and explains why SepSecS does not group together with its closest homolog, SepCysS, in the family tree of fold-type I PLP enzymes (12). Moreover, the proposed mechanism for SepSecS is similar to the one used by the bacterial SelA that also proceeds through a dehydroalanyl-tRNA^{Sec} intermediate (23). SepSecS therefore uses a primordial tRNA-dependent catalytic mechanism in which the PLP cofactor is directly involved, while using a tetrameric fold-type I architecture as the scaffold for binding the distinct structure of tRNA^{Sec}.

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Tiger Moth Jams Bat Sonar

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In response to sonar-guided attacking bats, some tiger moths make ultrasonic clicks of their own. The lepidopteran sounds have previously been shown to alert bats to some moths' toxic chemistry and also to startle bats unaccustomed to sonic prey. The moth sounds could also interfere with, or "jam," bat sonar, but evidence for such jamming has been inconclusive. Using ultrasonic recording and high-speed infrared videography of bat-moth interactions, we show that the palatable tiger moth *Bertholdia trigona* defends against attacking big brown bats (*Eptesicus fuscus*) using ultrasonic clicks that jam bat sonar. Sonar jamming extends the defensive repertoire available to prey in the long-standing evolutionary arms race between bats and insects.

The ability to pinpoint airborne insects in darkness, by echolocation (1), allowed bats to master nocturnal insectivory (2) and set the stage for the evolution of defensive countermeasures by insect prey (3). Some insects gained ears (4) and evasive maneuvering (5). Tiger moths (Lepidoptera: Arctiidae) developed the ability to click ultrasonically in response to attacking bats (6, 7). Decades of research on moth click defenses have led to three, not mutually exclusive, hypotheses regarding their function—startle (8), acoustic aposematism ("warning") (8–14), and sonar interference ("jamming") (15–17). When ultrasonic clicks are paired with unpalatable prey, bats learn to perceive clicks as a warning of unprofitability (8, 10–14). Moth clicks also startle inexperienced bats or bats that have not heard clicks for multiple days (8). Because bats habituate to startle quickly, its effectiveness as a defense requires clicking moths to be rare. This situation does not appear typical in nature (12).

Finally, moth clicks may disrupt the sonar of an attacking bat (15–17). Clicks might diminish a bat's acuity in determining target distance (17–19) or feign echoes from objects that do not exist (15). However, evidence that moth clicks can disrupt bat attacks by jamming sonar is lacking. One recent study found that moth clicks had no discernible effect on attacking bats unless clicks were paired with defensive chemistry (13). All previous studies, however, tested moths with relatively low duty cycles, or sound production per unit time (20). High-duty-cycle moth clicks, such as those of *Bertholdia trigona* (Fig. 1 and

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table S2), are more likely to jam bats (*17*, *18*), including the big brown bat (*Eptesicus fuscus*), which co-occurs with *B. trigona* in much of Central America and northward to Colorado, USA.

To determine whether the clicks of *B. trigona* startle, warn, or jam bats, we pitted *E. fuscus* against *B. trigona* in a flight room equipped with high-speed infrared video cameras and an ultrasonic microphone (*21*). The pattern of a naïve bat's success in catching clicking moths, when measured over several days, should differ for each of the three proposed moth click functions (Fig. 2A) (*13*). For a warning sound, bats initially capture and drop distasteful moths, then learn to abort attacks on hearing clicks (*8*, *13*, *22*). Alternatively, a bat could be initially startled by the clicks but would quickly habituate to the sounds (*8*, *23*). A jamming defense, however, would immediately deter attack-ing bats, and the effect of the defense would persist with time (Fig. 2A).

We trained three naïve juvenile *E. fuscus* (bats J1, J2, and J3) and one adult *E. fuscus* (bat A1) to capture tethered moths in a sound-attenuated flight room. Naïve bats allow us to control prior experience, whereas an adult bat's prior experience catching insects may allow it to better overcome moth defense. On each of nine consecutive nights, 16 tethered moths—4 *B. trigona*; 4 silent, palatable, size-matched noctuid novelty controls; and 8 silent, palatable wax moths (female *Galleria mellonella*, used in pre-experiment bat training)—were presented to each bat individually in a random order. Bats were allowed up to 1 min or five approaches for each moth. We presented unaltered *B. trigona* to the bats on nights one through seven and *B. trigona* lacking the ability to click (from tymbal abla-tion) on nights eight and nine. Seventy-eight percent of the *B. trigona* (87 of 112) responded acoustically to bat sonar. The few intact *B. trigona* that did not produce sound in response to bat attack were excluded from the analysis. We used contact with the moths as our measure of attack success. Palatability of *B. trigona* to each bat was taken as the proportion of captured moths eaten rather than dropped. We conducted feeding trials with silenced moths and four additional stationary bats to further assess the palatability of *B. trigona*. Finally, we analyzed echolocation recordings to measure bat behavioral response to moth clicks (*21*).

In stationary feeding trials with silenced moths, three bats regularly ate *B. trigona* (J4, 92%, n = 12; J5, 92%, n = 12; J6, 83%, n = 6), and one did not (J7, 0%; n = 8). Similarly, of the bats capturing moths in flight, three regularly ate *B. trigona* (J1, 94%, n = 16; J2, 50%, n = 14; A1, 89%, n = 18), and one did not (J3, 0%, n =12). The observed differences between individuals may reflect a greater reluctance of some individuals to incorporate new items into their diet, as has been reported for some birds (*24*). *B. trigona* appears palatable; the six bats ate the moths with no detectable ill effects. Because warning and jamming are not mutually exclusive defense functions (*25*), our ability to detect a jamming effect is diminished for the bat that rejected *B. trigona*—any moth avoidance could be caused by jamming, warning, or both. Thus, to unambiguously test the hypothesis of sonar jamming, we focus our analysis on the three bats that ate *B. trigona* in flight (J1, J2, and A1).

Each of the three bats contacted control moths over 400% more often than clicking *B. trigona* (Table 1 and movie S1). In contrast, bats contacted 100% of *B. trigona* when the moths' tymbals
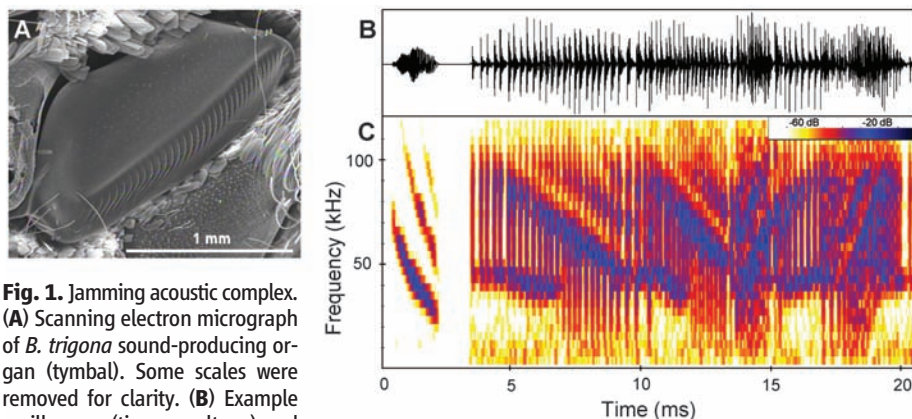
Fig. 1. Jamming acoustic complex. (**A**) Scanning electron micrograph of *B. trigona* sound-producing organ (tymbal). Some scales were removed for clarity. (**B**) Example oscillogram (time × voltage) and (**C**) spectrogram showing a simulated *E. fuscus* echo, reflected off of *B. trigona* at 1 m (left), and two overlapping *B. trigona* click modulation cycles (right). Echo and clicks are presented at average estimated intensities at the bat's location 1.0 m from the moth (*21*).

Fig. 2. Predicted and observed success rates for *E. fuscus* attacking moths using various defensive strategies. (**A**) Predicted bat attack success over successive nights for three proposed moth click functions: startle, aposematism, and jamming. (**B**) Observed bat attack success rates for three *E. fuscus* (J1, circles; J2, triangles; A1, squares) on *B. trigona* (closed symbols) and noctuid controls (open symbols). The solid line shows mean values for attacks on *B. trigona*. (**C**) Success rates of bats attacking intact (sound-producing) and silenced *B. trigona*. Means ± SEM. Some values were offset slightly for display.
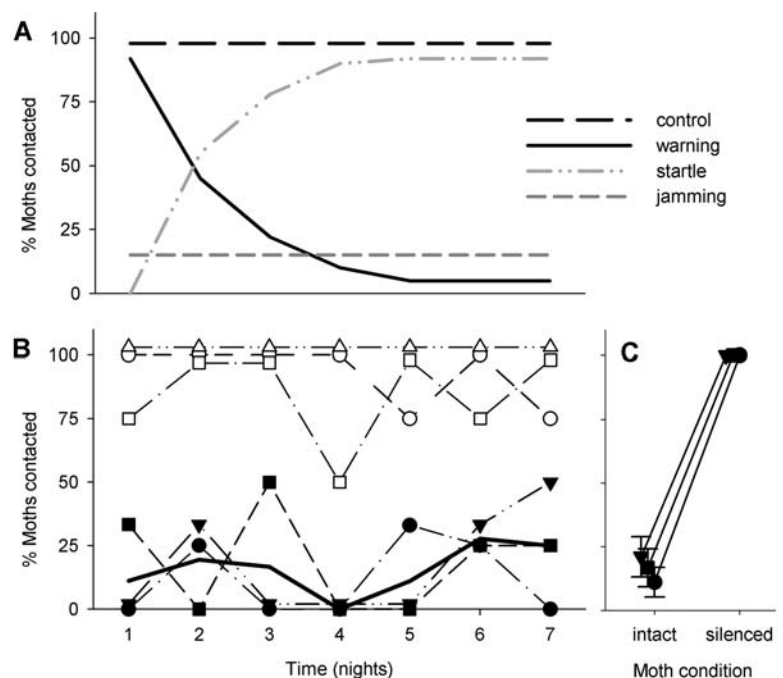
were ablated (Fig. 2C and movie S2). This confirms that the bats' poor performance on *B. trigona* was caused by the moth clicks. Rates of contact with sound-producing moths did not change during the experiment (Fig. 2B) [Friedman nonparametric analysis of variance (ANOVA), $\chi^2 = 2.63$, df = 6, $P = 0.85$]. Thus, prior learning was not required for the defense to be effective, nor did the bats habituate to the clicks. Bat contact rates did not improve on succeeding attacks on the same moth—attacks that often occurred within seconds of each other (table S1) (Friedman nonparametric ANOVA, $\chi^2 = 2.55$, df = 4, $P = 0.64$). This renders startle unlikely because nearly all mammals, including bats, habituate to startle (*8, 23*). Echolocation recordings (Fig. 3) showed bats persisting in attack attempts after onset of moth clicks to varying degrees throughout the experiment (fig. S1A). In contrast, bats in a previous study quickly learned to abort attacks on the noxious *Cycnia tenera* (fig. S1B) (*22*). This further suggests that the bats in our study did not learn to take the moth clicks as a warning—the bats continued attacks despite hearing the sounds. It also indicates that moth clicks are not generally aversive to bats [supporting online material (SOM) text]. Together, we

take the above results as strong evidence for a jamming function of the moth sound. The defense was effective immediately and persistently on juvenile and adult *E. fuscus* (Fig. 2B) that frequently tried to capture the clicking moths (fig. S1A) but had much difficulty doing so.

We also observed atypical echolocation behavior in response to *B. trigona* clicks. Each bat attacked all control moths by progressing through the approach, track, and terminal phases [e.g., (*26, 27*)]. However, in about a third (J1, 21%, *n* = 19; J2, 40%, *n* = 15; A1, 28%, *n* = 25) of attacks on *B. trigona*, the bat reversed the attack phase from track to approach or terminal to track, and then continued the attack (Fig. 3). This behavior did not diminish with experience (Friedman nonparametric ANOVA, $\chi^2 = 4.5$, df = 6, $P = 0.61$), as would be expected with startle. It appears to be a direct response of the bats to the sonar interference caused by the moth clicks.

The ultrasonic clicks of *B. trigona* appear well-suited for jamming echolocation (SOM text). Under all proposed jamming mechanisms, high-duty-cycle sounds made by highly developed tymbals (Fig. 1) should better jam echolocation (*15, 17–19*). Empirical evidence now supports

this prediction; the high-duty-cycle sounds of *B. trigona* jammed bats, whereas the low-duty-cycle sounds of *Euchaetes egle* were ineffective jamming signals under nearly identical conditions (*13*). The use of tiger moth sound for aposematic signaling requires only simple tymbal structure and appears widespread in the Arctiidae (*7, 10–13*). Thus, acoustic aposematism may have been a stepping-stone in the evolutionary development of a jamming signal, a unique acoustic countermeasure in the long-standing arms race between bats and their insect prey.

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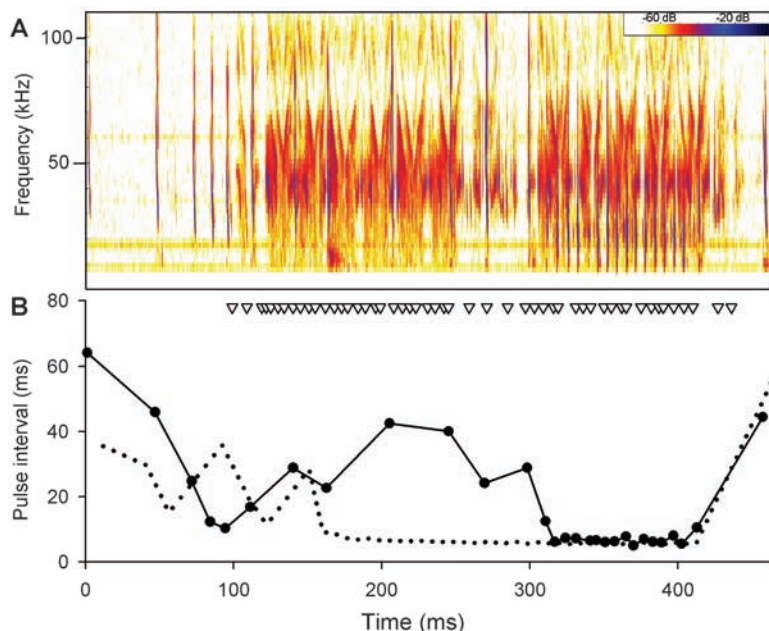
Fig. 3. Example echolocation attack sequence. (**A**) Spectrogram showing a big brown bat attacking a clicking *B. trigona*. (**B**) Pulse-interval graph of echolocation attack. Each circle connected by solid lines indicates the time of a sonar pulse and the duration of the following pulse interval. Open triangles indicate moth click half modulation cycles. The dotted line shows an attack on a control moth for reference.

Table 1. Contact rates of big brown bats on *B. trigona* and noctuid control moths.

Bat	B. trigona		Noctuid control		χ^2	df	P
	n	Percent contact	*n*	Percent contact			
J1	19	16	28	93	28.45	1	<0.001
J2	15	20	28	100	27.22	1	<0.001
A1	25	20	28	86	23.02	1	<0.001