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Floral Acoustics: Conspicuous Echoes of a Dish-Shaped Leaf Attract Bat Pollinators

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The visual splendor of many diurnal flowers serves to attract visually guided pollinators such as bees and birds, but it remains to be seen whether bat-pollinated flowers have evolved analogous echo-acoustic signals to lure their echolocating pollinators. Here, we demonstrate how an unusual dish-shaped leaf displayed above the inflorescences of the vine *Maragravia evenia* attracts bat pollinators. Specifically, this leaf’s echoes fulfilled requirements for an effective beacon, that is, they were strong, multidirectional, and had a recognizable invariant echo signature. In behavioral experiments, presence of the leaves halved foraging time for flower-visiting bats.

To attract pollinators, plants provide floral signals that contrast with their background. Bright colors that stand out against the green vegetation shorten search time for visually guided pollinators (1, 2), and there is some evidence that the echoes of bat-visited plants may similarly stand out against natural vegetation. Plants depending on bats for pollination and seed dispersal sometimes expose their flowers and fruits on long peduncles to facilitate approach and detection by echolocation (3, 4). Further, in some bat-pollinated flowers, characteristic echo features, such as prolonged duration and complex spectral composition, have been identified that may contain information about flower shape and orientation (5, 6). The only conclusive evidence for echo-acoustic signaling so far comes from a bat-pollinated epiphytic vine (Fabaceae, *Mucuna holtomii*), whose ripe flowers present a specialized concave petal that has strong echoes from many directions (7, 8). Removing the petal or modifying its echoes meant that the respective flowers were visited less often than unmodified flowers in the same inflorescence (7), which shows that the specialized petals of *M. holtomii* are local acoustic nectar guides. However, none of these studies showed that floral echo signals can attract bats and help them to find flowers faster or, in particular, that these signals can serve as an echo beacon that stands out among the clutter of vegetation echoes.

The compound echo of a plant consists of individual echoes from each leaf, and hence depends on the plant’s growth pattern (9). Most plants show a nonuniform arrangement of leaves and twigs; therefore, their echoes are largely random and, most important, change with the observer’s position (10). Among such unpredictable and changeable background echoes, any flower echo—however characteristic—will be a challenge to detect, particularly from a single position. Predictable echoes that appear similar regardless of observer position could stand out against a random and variable echo background. A flower with a spatially invariant echo is recognizable because it is the only object that does not change as a bat moves around and integrates echoes from several positions. To be an effective echo beacon, this recognizable signature should additionally be detectable over long distances (i.e., strong) to attract more distant bats (large “catchment volume”).

The Cuban bat-pollinated rainforest vine *Maragravia evenia* (Maragraviaceae) (11, 12) displays one (sometimes two) intriguing dish-shaped foliage leaves in association with its inflorescences (N = 12 examined; Fig. 1). The stalks of these leaves are twisted, bringing the leaf blade into an upright position, with the upper concave side facing approaching pollinators. The dish-shaped leaves (DL) differ significantly from foliage leaves (FL) in shape and, in particular, depth (Fig. 2, A and B; height by width by depth of DL: 51 ± 19 by 35 ± 3 by 13 ± 5 mm versus FL: 74 ± 5 by 28 ± 3 by 3 ± 1 mm, respectively, mean ± SD; N = 12 FL, 10 DL; t10 = 7.245; P < 0.001). The IRs of a foliage leaf consist of two reflections, one from either side of the leaf blade (light and dark blue arrows in Fig. 2, C and G). As the leaf is turned, one side gets closer while the other retreats from the sound source, generating the X-shaped pattern in Fig. 2G. The dish-shaped leaf showed the same X-shaped pattern (Fig. 2H) originating from its left and right edges (blue arrows in Fig. 2, D and I), but, in

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Fig. 1. Flowering inflorescence of *M. evenia*. (A) Dish-shaped leaf. (B) Ring of flowers, most of them in the male phase with anthers shedding pollen. (C) Cup-like nectaries. Image provided by R. Mangelsdorff.
addition, it had two higher-amplitude peaks from within its cavity—a single reflection from its center and a double reflection bouncing off both sides (orange and red arrows, respectively, in Fig. 2D and between 1.25 and 1.35 ms in Fig. 2H). These two peaks show a distinct temporal separation of 41.7 ± 6.1 µs (Fig. 2H) and were present over a wide range of angles (61.3 ± 11.9°). Within this angular range, the temporal separation displayed was remarkably constant across individual leaves (mean SD, 5.7 ± 1.4 µs). These reflection pathways (Fig. 2D) are the same as in hollow hemispheres (13), and the temporal separation closely matches that calculated for a hemisphere with the leaf’s cross-section radius [difference between measured and calculated values: 3.4 ± 2.2 µs, mean ± SD; N = 6 DL; formula in (13)].

Through interference, the time separations between all amplitude peaks found in the IR determine which frequencies in the echo are enhanced and which are cancelled. For the foliage leaf, the changes in time separation between the two main peaks in the IR (X pattern in Fig. 2G) result in drastic changes in echo spectra, with many steep interference bands in the directional spectra (Fig. 2I). This means foliage leaves reflect different echo spectra even for small changes in direction, as expected for background vegetation (9, 10). In contrast, directional spectra of dish-shaped leaves were dominated by the two central amplitude peaks (orange and red arrows in Fig. 2H), with almost constant time separation. As a consequence, interference wavelengths were more or less constant, giving the echoes a spectral signature that changed only very moderately over a wide range of directions (Fig. 2J), which should be conspicuously constant to a passing bat. In summary, dish-shaped leaves have a large catchment volume and reflection paths depicted in (C) and (D) in the same colors. (B) n a t u r a l D L (H); s e e c o l o r b a r t o t h e l e f t f o r s p e c t r a l t a r g e t s t r e n g t h v a l u e s. (A) I m a g e s o f a F L (A) a n d a D L (B).

To investigate whether the echoes of dish-shaped leaves do indeed help bats find flowers, we trained nectar-feeding bats (Glossophaga soricina, Phyllostomidae) to search for a single small feeder (2.5-cm diameter, hidden within an artificial foliage background of 2.2 m²) and then measured the time the bat needed to find the feeder at changing positions. The feeder was presented on its own or with a replica of either a foliage leaf or a dish-shaped leaf. Each feeder type was randomly tested once at each of the 64 positions provided within the artificial foliage background (15). Search times were longest for all bats when the feeder was presented on its own (23.2 ± 4.4 s) and were slightly, but not significantly, shorter when a replica of a foliage leaf was added (22.0 ± 5.6 s). However, a dish-shaped leaf replica above the feeder always reduced search times (12.3 ± 1.3 s, mean ± SE; N = 3 bats; generalized linear model with feeder type as fixed factor and bat as random factor; feeder type: F_{2,4} = 16.783, P = 0.011; bat: F_{2,4} = 6.230, P = 0.059; post-hoc Tukey: DL versus FL: P = 0.020; DL versus no leaf: P = 0.013; FL versus no leaf: P = 0.842). The dish-shaped leaf reduced search time by 46.3 ± 8.6% relative to that for the feeder presented solely, whereas the foliage leaf only reduced search time by 6.0 ± 7.0% (mean ± SE) (Fig. 2, K and L).

Feeder detection was based on echolocation in this experiment; thus, these results demonstrate that the dish-shaped leaf’s echoes render it

Fig. 2. Acoustic characterization of foliage leaves (FL, left column) and dish-shaped leaves (DL, right column) of M. evenia and their effects on the bats’ search time. (A and B) Images of a FL (A) and a DL (B). Scale bar, 1 cm. (C and D) Horizontal cross sections of both leaf types, with main echo reflection paths for angles of sound incidence of 20° and 0°. In (D), a circle (gray area) was fitted to highlight the leaf’s spherical shape. (E and F) Mean spectral target strength of replicas of FL (E) and DL (F) as a function of angle. SD given in green (N = 6 each). Black lines show mean spectral target strengths of natural leaves (N = 5 FL, 3 DL). (G and H) Directional pattern of the impulse response of one natural FL (G) and one natural DL (H); see color bar to the left for amplitudes. Arrows indicate amplitude peaks caused by the reflection paths depicted in (C) and (D) in the same colors. (I and J) Directional power spectra corresponding to (G) and (H); compare color bar to the left for spectral target strength values. (K and L) Reduction (mean ± SE) in search time of three nectar-feeding bats (G. soricina) trained to find a single feeder hidden in artificial vegetation when a FL (K) or a DL (L) replica was presented above the feeder. Reduction is given relative to the mean search time for the feeder on its own; each bar represents 64 trials.
Initiation Complex Structure and Promoter Proofreading

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The initiation of transcription by RNA polymerase II is a multistage process. X-ray crystal structures of transcription complexes containing short RNAs reveal three structural states: one with 2- and 3-nucleotide RNAs, in which only the 3′-end of the RNA is detectable; a second state with 4- and 5-nucleotide RNAs, with an RNA-DNA hybrid in a grossly distorted conformation; and a third state with RNAs of 6 nucleotides and longer, essentially the same as a stable elongating complex. The transition from the first to the second state correlates with a markedly reduced frequency of abortive initiation. The transition from the second to the third state correlates with partial "bubble collapse" and promoter escape. Polymerase structure is permissive for abortive initiation, thereby setting a lower limit on polymerase-promoter complex lifetime and allowing the dissociation of nonspecific complexes. Abortive initiation may be viewed as promoter proofreading, and the structural transitions as checkpoints for promoter control.

The initiation of RNA polymerase II (Pol II) transcription is a focal point of cellular regulation. Initiation proceeds through multiple stages, each of which may be subject to intervention by regulatory factors. Stages so far recognized entail synthesis of transcripts with lengths of about 5, 10, and 25 nucleotides (nt) (1). Transcripts of less than 5 nt are unstable, resulting in frequent “abortive initiation.” At about 10 nt, interactions with general factors are disrupted, resulting in “promoter escape.” The initiation process concludes when, at a transcript length of about 25 nt, a transition is made to a stable “elongation complex.” Here, we report structures of Pol II with short transcripts that illuminate some of the earliest events of transcription initiation.

Previous biochemical studies showed that the synthesis of a 3- to 4-nt transcript confers a degree of stability, referred to as “escape commitment” (Fig. 1), revealed by a reduced incidence of abortive initiation and by the end of a requirement for adenosine triphosphate (ATP) hydrolysis for maintenance of a transcription “bubble” (2–8). A transcript length of about 7 nt induces partial collapse of the transcription bubble, which coincides with the start of promoter escape (9). An 8-base pair RNA-DNA hybrid is necessary and sufficient for the formation of a stable transcribing complex (10). Transcript “slipping,” in which a short RNA dissociates from the template DNA and reanneals with a repeating element upstream, correlates with hybrid strength as well (11, 12).

Interactions with both Pol II and general transcription factors modulate transcript stability.

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