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Evasive response to ultrasound by the crepuscular butterfly *Manataria maculata*

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Abstract The crepuscular nymphalid butterfly *Manataria maculata* was studied in Monteverde cloud forest, Costa Rica, during the dry season reproductive diapause. *M. maculata* has ears in the form of Vogel's organs located near the base of the forewings. Its behaviour in response to bursts of ultrasonic pulses (26 kHz, 110 dB SPL at 1 m) was condition-dependent. At dusk and dawn the sound consistently elicited evasive responses, similar to those of moths, in flying individuals. In contrast day-roosting individuals always remained motionless although they were alert to other stimuli. The daily movements between day- and night-roosts coincided in time and light intensity with the activity of insectivorous bats. This is the first reported case of ultrasonic hearing connected to evasive flights in a true butterfly (Papilionoidea). It strongly supports the idea that echolocating bats were involved in the evolution of hearing in butterflies.

Introduction

Ultrasound-sensitive ears are common in Lepidoptera (moths and butterflies) and many other insects and provide an early warning system against echolocating bats. The ears are located on various parts of the body and also differ morphologically in the various taxonomic groups. In the Lepidoptera, ears have evolved independently at least eight times, most probably in response to predation pressure from bats (Roeder 1967; Fullard 1998; Miller and Surlykke 2001).

Alar tympanic organs called Vogel's organs occur near the base of the wing veins of some butterflies of the

family Nymphalidae, including *Heliconius* sp. (Heliconiinae) (Swihart 1967), *Erebia* spp. (Satyrinae) (Ribaric and Gogala 1996) and *Hamadryas* spp. (Biblidinae) (Yack et al. 2000). These organs respond to low frequency sound (1–2 kHz) but are insensitive to ultrasound. We here report the first case of ultrasonic hearing in a true butterfly, the neotropical nymphalid *Manataria maculata* (Satyrinae). This species is crepuscular (DeVries 1987; Stevenson and Haber 2000), unlike most other butterflies (Fullard and Napoleone 2001), and we speculate that it may be subject to natural selection from bats.

Natural history of *Manataria maculata*

M. maculata is a large forest nymph (forewing length 40–45 mm). Its brown coloration matches the typical day-roosts in the shade on or near the ground (DeVries 1987). In Costa Rica, *M. maculata* reproduces in deciduous forest on the Pacific lowland during the wet season (April–May). In July and August recently emerged butterflies migrate east to the evergreen forests on the continental divide, including the Monteverde cloud forest, where they remain in reproductive diapause until the following April, before returning to the lowlands. The butterflies feed in the daytime on rotting fruit and tree sap, but during the diapause this occurs only sporadically (Stevenson and Haber 2000).

The behaviour of *M. maculata* is unusual among butterflies and rather like that of moths. Most flight activity, including the migrations, takes place during 15–20 min at dusk and dawn. In Monteverde, groups of 10–50 *M. maculata* typically leave their communal day-roosts on the ground in a coordinated manner at dusk and spread out to individual night-roosts in the tree canopy. They return to the day-roosts one by one at dawn (Stevenson and Haber 2000). Because *M. maculata* usually does not fly until after sunset, and therefore cannot warm up by using radiant heat from the sun, they typically vibrate their wings before take-off, thereby

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raising their body temperature to 7–10°C above the ambient (Stevenson and Haber 2000).

Because of its crepuscular behaviour, we suspected that *M. maculata* would be exposed to insectivorous bats, of which there are some 30 species in Monteverde (Timm and LaVal 2000). We hypothesized that bat predation could have selected for an ultrasound-sensitive hearing organ in these butterflies.

Materials and methods

We observed *M. maculata* near Estación Biológica on the Pacific side of the Monteverde cloud forest at 1,500–1,600 m a.s.l. in December 2000. The trade wind blew strongly during the study and brought frequent rains, but the observations were made in clear weather, which occurred intermittently. Butterflies were exposed to ultrasonic pulses (26 kHz, 110 dB SPL (rms) at 1 m, second harmonic attenuated >30 dB; Rydell et al. 1997) from an electronic “dog whistle” which was triggered manually. For controls, we pressed the opposite side of the whistle, so that no sound was produced. The dog whistle is a simple device, but produces a sound that is similar to that of aerial-hawking bats in frequency and intensity (but not in the frequency vs. time structure), and elicits normal evasive responses from other species of Lepidoptera (Rydell et al. 1997).

Experiments on flying *M. maculata* were made during the evening exodus of groups of butterflies or during the return of single individuals in the morning. Tests were also made on roosting groups during the day. The sound tests were made at various distances, but following each test the distance was measured to the nearest metre. Tests were centred on three different roosts, each containing 10–40 individuals. To minimize habituation effects, we avoided testing individuals from the same roost on successive days.

To determine the incident light levels during periods when the butterflies were active, we used a light meter (Gossen Mastersix) with the diffuser on and directed towards the open sky. A heterodyne bat detector (Pettersson D-920) tuned to approximately 34 kHz was used to monitor the activity of bats near the butterfly roosts.

Results

M. maculata left their communal day-roosts as cohesive groups at 1645–1715 hours at incident light levels of 7–70 lx. They returned, usually one by one, at 0545–0630 hours at light intensities of 2–94 lx. Unknown species of insectivorous bats, i.e. high-intensity echolocators, were heard commuting and/or foraging on four occasions during these time and light intervals.

M. maculata responded to the ultrasound by evasive flights. These were very distinct and consisted of either a rapid dive or a loop usually 0.3–2 m deep, sometimes followed by an upward flight that resumed the original path. In some cases the reaction consisted of a distinct lateral deviation away from the sound source. Several evasive flights of the same or different types sometimes occurred in rapid succession. It seems very likely that a continuous pulse train (from a real bat) would have brought the insect to the ground, although we did not observe this. When groups of butterflies (>10 individuals) were tested, most individuals usually reacted simultaneously.

Table 1 Frequency of evasive reaction of free-flying *Manataria maculata* in response to ultrasound (26 kHz). The tests were made at distances of 0.5–5 m. For groups of butterflies reaction was scored when >50% of the individuals responded simultaneously

Category	<i>n</i>	% responding	<i>P</i> (χ^2 test)
Single individuals			
Test	192	91.1	<0.001
Control	111	3.6	
Cohesive groups (>10 individuals)			
Test	15	93.3	<0.001
Control	11	0.0	

The evasive reactions occurred in response to the sound and not to anything else, since the controls (no sound) usually did not elicit any reaction (Table 1). This applied to both individuals and groups. The frequency of reaction of individual butterflies remained high (91%, *n*=212) up to a distance of 10 m from the sound source but then declined (69%, *n*=102, at 11–15 m and 35%, *n*=23, at 16–17 m; χ^2 test, *P*<0.001).

Groups of roosting *M. maculata* did not respond in any visible way to ultrasound during the day, not even when the sound was delivered at very close range (<0.5 m). However, they were alert and easily flushed when touched and also when rapid movements occurred near the roost.

Examination of six *M. maculata* from Costa Rica revealed that the Vogel’s organ is located at the base of the subcostal and cubital veins on the underside of each forewing. It is morphologically similar to that of other nymphalids (Vogel 1912; Scoble 1992; Yack et al. 2000). Microscopic examination of the thorax and abdomen before and after maceration in hot KOH did not reveal any structures resembling known lepidopteran ears or air chambers that may have been connected to such ears (Cook and Scoble 1992; Maes 1985). Likewise there were no modifications of the mouthparts, indicating ears like those of sphingids (Göpfert and Wasserthal 1999).

Discussion

Manataria maculata possesses what seems to be a highly developed and functional defence against insectivorous bats. It is based on ultrasonic hearing that triggers defensive reactions similar to those of nocturnal moths (Roeder 1967). Resting *M. maculata* did not respond to the simulated bat calls, although they were alert to other kinds of stimuli. This makes sense because remaining motionless on the ground is almost certainly the most appropriate response when threatened by a bat (e.g. Jensen et al. 2001). We cannot completely exclude the possibility that *M. maculata* has ears other than Vogel’s organs, although we find this very unlikely.

Since we measured the distance to the responding butterflies and we know the intensity of the sound (110 dB SPL), we can estimate the hearing threshold of the

butterflies. Assuming a geometrical spreading loss of 6 dB per doubling of distance and an atmospheric attenuation of 1 dB per metre (at 30 kHz and 50% relative humidity; Lawrence and Simmons 1982), the behavioural hearing threshold was roughly 70 dB SPL, which is comparable to that of other lepidopterans (Fullard 1979; Skals and Surlykke 2000; Yack et al. 2000).

Our findings have implications for the evolution of the butterflies, although the interpretation is complicated. It seems likely that the Hedyloidea is the sister group of the skipper butterflies (Hesperoidea) and the true butterflies (Papilionoidea), and that from the true butterflies line have branched off in turn the Papilionidae, the Pieridae and the Lycaenidae (+ Riodinidae) and the Nymphalidae as terminal sister groups (Kristensen 1976; De Jong et al. 1996). The Hedyloidea are nocturnal butterflies (Scoble 1986; Aiello 1992) with ultrasound-sensitive ears (Scoble 1992) that are used in defence against bats (Yack and Fullard 1999). Hence, ears occur in the Hedyloidea and in at least some Nymphalidae, but apparently not in the Hesperidae, Papilionidae, Pieridae and Lycaenidae. The ears of the Hedyloidea are totally different from those of Drepanidae and Geometridae and, therefore, ears probably evolved at least twice within the butterfly clade; in the Hedyloidea and then again in a nocturnal or crepuscular form of Nymphalidae.

The alternative hypothesis is that the ears of the Hedyloidea and the Nymphalidae are homologous (Yack and Fullard 1999), having evolved in a common ancestor of the Hedyloidea + Hesperoidea/Papilionoidea clade. This implies, however, that the ears have been lost at least four times independently; in the Hesperidae, Papilionidae, Pieridae, Lycaenidae and perhaps also in various Nymphalidae. This hypothesis involves more changes in character state than the first hypothesis and therefore seems less parsimonious as an explanation.

The systematic position of *Manataria* is among the Satyrinae within the Nymphalidae (Miller 1968; Brower 2000). It remains unknown whether the low-frequency ears of other satyrines such as *Erebia* spp. are simply degenerate bat detectors, or whether they have evolved in a different context. This also applies to *Heliconius* (Heliconiinae) and *Hamadryas* (Biblidinae). In the latter genus the ears are probably used in communication (Yack et al. 2000). This is also the case in some moths, including species of Arctiidae, Noctuidae and Pyralidae, where ultrasonic communication most probably evolved secondarily, following the evolution of hearing as a defence against bats (Conner 1999).

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