

— Short Communication —

THE FLORAL BAT LURE DIMETHYL DISULPHIDE DOES NOT ATTRACT THE PALAEO-TROPICAL DAWN BAT

Gerald G. Carter* and Alyssa B. Stewart

Department of Biology, University of Maryland, College Park, MD 20742, USA

Abstract—In the neotropics, dimethyl disulphide (DMDS) is innately attractive to flower-visiting bats, and acts as a powerful bat lure in the scent bouquets of many bat-pollinated flowers. At first, DMDS appeared to be part of a general bat pollination syndrome. However, DMDS is absent in many bat-pollinated flowers of West Africa, and it is unclear whether palaeotropical flower-visiting bats are also attracted to it. Furthermore, DMDS was previously observed in neotropical, but not palaeotropical, populations of *Ceiba pentandra* (Malvaceae, Bombacoideae). We tested for an attraction to DMDS in the most common flower-visiting bat in Thailand, the dawn bat *Eonycteris spelaea*. We gave bats choices of *Ceiba pentandra* flowers, where one random flower was scented with DMDS. Rather than preferring the DMDS-treated flower, 21 of 22 bats chose an untreated flower, showing no attraction to DMDS. Alongside past evidence, this result suggests that the role of DMDS in bat pollination syndromes may result from an adaptive convergence that is limited to the neotropics. This hypothesis could be tested through comparative studies of (1) attraction across bats, (2) floral DMDS presence across bat-pollinated plants in Asia, and (3) floral DMDS measures across New and Old World populations of *Ceiba pentandra*.

Keywords: bat pollination, *Ceiba pentandra*, dimethyl disulphide, *Eonycteris spelaea*, floral scent, olfaction

INTRODUCTION

The odourous sulphur compound, dimethyl disulphide (DMDS), is a powerful attractant of neotropical nectarivorous bats (*Glossophaga* sp., von Helversen et al. 2000; *Anoura* sp., N. Muchhala, pers. comm.). Flower-visiting bats use DMDS as a beacon to find flower locations and to locate the nectar reward within the flower. Captive-born long-tongued bats *Glossophaga soricina* with no previous experience of the scent will hover and stick their heads into tubes of DMDS, which demonstrates that their attraction to the scent is innate (von Helversen et al. 2000; Carter et al. 2009).

DMDS appears to have evolved independently in the scent bouquets of many neotropical bat-pollinated flowers (Knudsen et al. 1995; Bestmann et al. 1997; von Helversen et al. 2000), but its evolutionary origin as a bat lure is unclear. Given that DMDS is attractive to the males of some mammalian species (e.g. Singer et al. 1976), one hypothesis is that its presence in flowers represents sensory exploitation of olfactory communication in bats or other mammals (von Helversen et al. 2000). DMDS may also have originally attracted flies (Jürgens et al. 2006; Shuttleworth & Johnson 2010; Stensmyr et al. 2002), and then later became innately attractive to bats. Based on early reports of floral odours in both the palaeotropics and neotropics, von Helversen *et al.*

(2000) suggested that the peculiar and unpleasant odour of DMDS and other sulphur compounds might be common to bat-pollinated flowers in general. However, later observations suggest that DMDS may only attract bats in the Neotropics. In West Africa, Petterson et al. (2004) sampled the scent composition of seven bat-pollinated flowers and found that only the baobab *Adansonia digitata* (Malvaceae, Bombacoideae), possessed more than a trace amount of DMDS.

Perhaps most remarkable is the case of the silk-cotton tree *Ceiba pentandra* (Malvaceae, Bombacoideae), which is pollinated by bats on both hemispheres. In Central America, *C. pentandra* flowers possess a substantial amount of DMDS (von Helversen et al. 2000), but in West Africa, they contain no detectable DMDS (Petterson et al. 2004). In India, the short-nosed fruit bat (*Cynopterus sphinx*) sometimes feeds on nectar but it does not prefer the scent of DMDS over other odours or scentless controls (Elangovan et al. 2006). These two pieces of evidence suggest that DMDS may only be a component of the pollination syndrome for bats in the neotropics but not the palaeotropics.

Nectarivory in bats arose independently in the neotropical Phyllostomidae (subfamily Glossophaginae) and the palaeotropical Pteropodidae, lineages that diverged about 56 mya (Fleming et al. 2009; Jones et al. 2005; Teeling et al. 2005). The evolutionary origins of DMDS as a bat lure could therefore be clarified further by testing if an innate attraction to DMDS occurs in palaeotropical bats. Here, we tested whether the dawn bat (*Eonycteris spelaea*), a common palaeotropical nectarivorous bat, is attracted to DMDS.

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*Corresponding author: gcarter@umd.edu

MATERIALS AND METHODS

We conducted 22 preference tests where single *E. spelaea* bats chose from a row of three ($N = 2$) or four ($N = 20$) *Ceiba pentandra* flowers. One flower was randomly scented with DMDS (Sigma-Aldrich, Inc, USA), and the others acted as untreated controls. We caught the bats ($N = 22$) in mist nets near Hat Yai, Thailand from 15 to 21 December 2014, and fed them with sugar water. The next night, we placed each bat in a $30 \times 36 \times 50$ cm plastic-grid test cage. We placed all bats at the same starting point in the test cage. On the opposite side of the test cage we created a linear array of flowers spaced apart 5 cm. Each of the six arrays we made contained flowers from the same *C. pentandra* tree ($N = 4$ trees). To treat flowers, we either soaked 200 μ L DMDS directly into one flower (3 trials) or pipetted it in a 0.5 mL tube positioned just below the flower (19 trials). The 0.5 mL tube's opening was directly below the flower's opening, but the odour tube remained outside the cage. After each trial, we used a fan to clear odours from the array for at least 3 min. To score choices, we used an infrared spotlight and a Sony Nightshot Camera to see which flower the bat first probed with its snout. Trials lasted 1–30 min. Using two-way binomial tests, we compared the selection rate of *E. spelaea* to the expected rate under random chance (25.73 %).

We also used a two-way binomial test to compare the *E. spelaea* selection rate to expected rates based on choices by the neotropical bat *Glossophaga soricina*. To calculate this expected rate, we used unpublished data from a pilot test that was most similar in design to this test. At the Montreal Biodome (see Carter et al. 2009) 19 naive captive-born *G. soricina* chose from arrays of four tubes, one randomly marked with the same amount of DMDS as used in the tests above. Under these similar conditions, the mean DMDS choice rate across *G. soricina* bats weighted by number of visits per bat ($N = 1$ to 33) was 50.46% (chance = 25 %, 95% C.I. = 38–63 %, Carter et al. 2009, unpublished data).

RESULTS AND DISCUSSION

Eonycteris spelaea chose the flower marked with dimethyl disulphide in only one of 22 trials (selection rate = 4.5 %), fewer times than expected based on either random chance ($P = 0.025$, Fig. 1) or the *G. soricina* preference ($P < 0.0001$). This result suggests that *E. spelaea*, unlike *G. soricina*, is not innately attracted to DMDS. Instead, we found evidence that the bats avoided the DMDS-treated flower, presumably because the DMDS masked the flower's natural odour. This adds to a growing body of knowledge on how bat pollination syndromes differ between the neotropics and palaeotropics (Pettersson et al. 2004; Fleming et al. 2009).

Given that *C. pentandra* flowers possess DMDS in Central America (von Helversen et al. 2000), but not West Africa (Pettersson et al. 2004), one possibility is that floral DMDS is present only in neotropical populations. Evidence suggests that mutations in floral traits can lead to rapid evolutionary shifts in pollination syndromes (Rosas-Guerrero et al. 2014). Shuttlesworth and Johnson (2010)

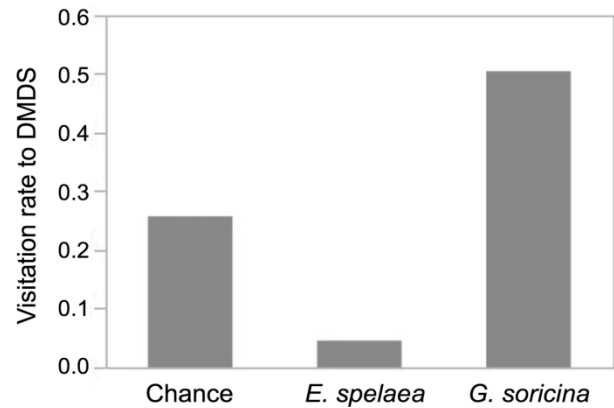


FIGURE 1. Observed and expected dimethyl disulphide selection rates. Rates of choices (probing with snout) to dimethyl disulphide odour cues are shown for the palaeotropical bat *Eonycteris spelaea*, alongside the expected levels based on chance and choices by the neotropical bat *Glossophaga soricina*.

showed that mutations leading to the presence and absence of DMDS alone have likely led to evolutionary transitions among insect pollinators. They studied four closely related species of *Eucomis* (Hyacinthaceae) that are pollinated by either wasps or carrion flies (which are attracted to DMDS). All four *Eucomis* species have flowers of similar colour, nectar, and morphology, but differ in scent compounds. In a field test, they experimentally manipulated floral scents and demonstrated that the presence of dimethyl disulphide and dimethyl trisulphide led to shifts from wasp to fly pollinators. Hence, evolutionary shifts between pollinators can occur based on DMDS alone, without changes in floral shape, colour or nectar. Similar co-evolutionary transitions may also have occurred for palaeotropical and neotropical flower-visiting bats.

Comparative studies would provide great insight into the coevolution of bat nectarivory and floral DMDS. First, to determine the extent of innate DMDS attraction in bats, it would be informative to test other captive-born or wild palaeotropical pteropodids as well as the neotropical Lonchophyllinae—a lineage which may have evolved their nectarivory independent of the Glossophaginae (Griffiths 1982; Datzmann et al. 2010; see also critical review by Dávalos et al. 2012). Second, to confirm a dichotomy in floral DMDS between neotropical and palaeotropical plant species, one could test for DMDS in other bat-pollinated flowers in Asia. Finally, the hypothesis of a neotropical versus palaeotropical dichotomy in floral DMDS within *C. pentandra* could be tested by mapping the occurrence of floral DMDS across the species range.

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