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An airborne sex pheromone in snakes

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Most reptile sex pheromones so far described are lipid molecules too large to diffuse through the air; instead, they are detected via direct contact (tongue-flicking) with another animal's body or substrate-deposited trails, using the vomeronasal system. The only non-lipid pheromone reported in snakes involves courtship termination in red-sided gartersnakes (*Thamnophis sirtalis parietalis*): males that encounter copulatory fluids cease courtship, presumably reflecting the futility of courting an already-mating female. Our field experiments at a communal den in Manitoba show that this pheromone can work via olfaction: courtship is terminated by exposure to airborne scents from mating conspecifics, and does not require direct contact (tongue-flicking). Hence, the sexual behaviour of reptiles can be affected by airborne as well as substrate-bound pheromones.

Keywords: chemical communication; reproductive tactics; reptile sociality; sexual selection

1. INTRODUCTION

In sexually reproducing species, an individual's fitness can be enhanced by the ability to rapidly detect the sex and reproductive state of conspecifics [1–3]. A range of sensory modalities can be used to obtain that information, depending on the species' biology. For example, visual cues often are important sex-identifying traits in taxa with high visual acuity (such as many birds and lizards), whereas scent is more important in taxa with sophisticated chemosensory abilities but relatively poor eyesight (such as many fossorial mammals and snakes [4,5]). A single species may detect a single type of cue using more than one sensory system. In snakes, for example, scent can be detected either via olfaction (airborne cues, taken in through the nostrils) or vomerolfaction (substrate-deposited cues, accessed through the forked tongue and vomeronasal system [5]). Although snakes use olfaction to obtain information about prey items [6], their sexual behaviour is driven primarily by the vomeronasal system. Cutting olfactory nerves does not affect courtship responses by male snakes, whereas cutting vomeronasal nerves prevents courtship [7–10]. The sex-identifying chemical cues so far identified in snakes are large epidermal lipids, incapable of diffusing through the air [5] or water [11]. Thus, snakes must tongue-flick other snakes, or substrate-deposited trails, to determine another animal's sex and reproductive state [12–15]. In lizards

also, males appear to determine the sex of conspecifics based on substrate-borne and not airborne cues [16].

If males need to rapidly assess the sex and reproductive condition of conspecifics, we might expect them to use whatever cues are available. Can male snakes detect sex pheromones not only by vomerolfaction, but also by olfaction? For this to be possible, the molecules involved must be small enough to diffuse through air. Observations on gartersnakes have identified an unusual sex pheromone that might fit this criterion. Female gartersnakes are simultaneously courted by multiple males, but the males cease courting as soon as one of them begins to copulate with the female. The cessation of courting is mediated by copulatory fluids; females smeared with those fluids cease to attract courtship [14,17]. The dilute watery appearance of the ejaculate suggests that it might diffuse through the air, and thus constitute an airborne sex pheromone. We conducted experimental trials to test that possibility.

2. MATERIAL AND METHODS

A limestone quarry near Inwood, Manitoba (50°7'N 97°9'W) contains a den with more than 30 000 red-sided gartersnakes (*Thamnophis sirtalis parietalis*) that court and mate in early spring [18]. As females emerge from the den they are surrounded by groups of males, each aligning his body to hers, and vigorously chin-rubbing and caudocephalic-waving. These snakes virtually ignore human presence, so we were able to sit in the den area, holding a recently emerged (unmated) female by the tail while she was courted by multiple males. To provide airborne cues, we used a portable aquarium pump to move air through plastic tubing into an empty margarine container (10 cm diameter and 3 cm high), and out the other side by another length of tubing. The open end of the tube was placed level with and 2 cm in front of a courting male's snout such that air blew across his snout at a 45° angle. We used a total of seven treatments. Three of these were controls (disturbance control (no tube or box); box empty, with or without the pump switched on) and four involved snakes within the margarine container (an unmated female; a male; a female plus a male; or a mating pair). All of these experimental snakes were collected at the den immediately prior to the trials. We conducted 20 replicates of each treatment (incorporating four different sets of odour-donating snakes in the relevant treatments) in randomized order. Our response variable was the duration of time the male continued courting, up to a maximum of 20 s (trials in which the male continued courting past this time were scored as 20 s). We predicted that if males can detect the copulatory pheromone through the air, the response to a mating pair of snakes inside the container (i.e. to the presence of copulatory fluids) should differ from all other treatments. The data were non-normally distributed, so were analysed with a Kruskal–Wallis test, with treatment as the factor and duration of courtship post-exposure as the dependent variable.

3. RESULTS

Treatment strongly affected the duration of courtship ($H_6 = 38.89$, $p < 0.0001$). Cessation of courtship was commonly observed in the 'mating pair' treatment, but not in any of the others (figure 1).

4. DISCUSSION

Male snakes in our study population use epidermal lipids (methyl ketones) not only to determine the sex of another snake [5,19], but also to evaluate the other animal's body length and condition [20]. The current study reveals a role for airborne pheromones as well, and constitutes the first example of this phenomenon in snakes.

A male gartersnake's use of airborne scent cues makes sense in light of the mating system. Courtship is

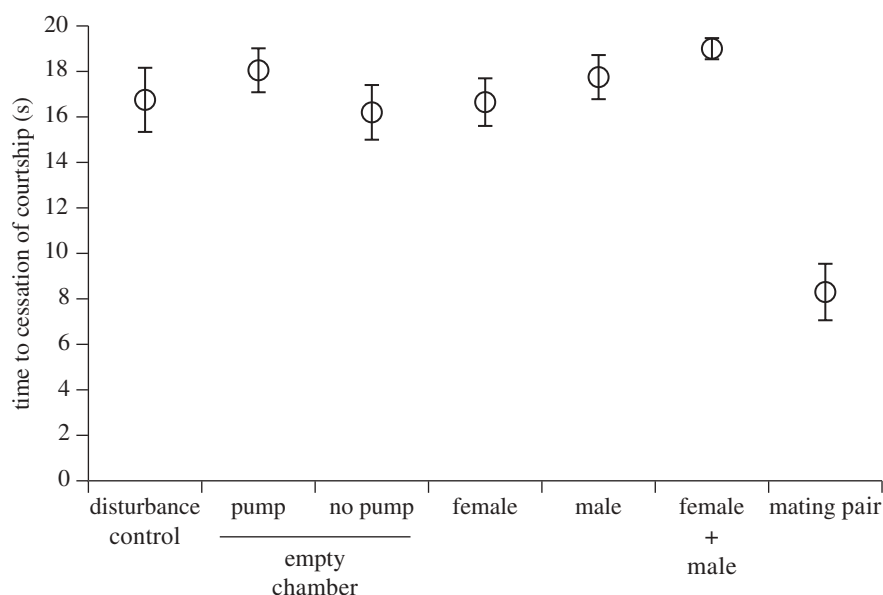


Figure 1. The effect of airborne scents on the duration of courtship by free-ranging male gartersnakes, at a communal den in Manitoba. Courting males ($n = 20$ per treatment) were exposed to scents of other snakes, and controls; the only treatment that curtailed courtship was airborne scent from a mating pair of snakes. The graph shows mean values and s.e.

energetically expensive [15], and continuing to court an already mating female is futile because mating males deposit a gelatinous plug that occludes the female's cloaca, preventing her from re-mating [17]. Thus, a male could benefit from rapidly detecting that mating is occurring, so that he can move on to another female. However, it may be difficult for a male to obtain evidence of mating. Any unmated female is surrounded by a dense group of courting males, such that a given male has access to only a small part of the female's epidermis; and any changes in female skin lipids post-mating probably occur over a timeframe of hours or days rather than minutes. The male potentially could obtain direct visual and/or chemosensory evidence of copulation by inspecting the female's cloacal region, but this would be difficult because the female's tailbase is covered by males. Airborne cues from the ejaculate offer another—and easier-to-access—indication of copulation. Previous experimental work has shown that copulatory fluids contain the cue that terminates male courtship [17]; and the current study shows that this cue can be transmitted via olfaction.

Our results reinforce the complex and multifactorial way in which reproducing male snakes assess the attributes of potential mates. Although substrate-bound lipids undoubtedly play a central role in mate recognition in snakes, males also exploit a range of other cues in making their reproductive decisions. Those additional cues include visual and thermal characteristics of the female, and courtship by other males [11,21], as well as airborne scents (current study). The vomeronasal system thus plays a dominant but not exclusive role in sexual communication within snakes. Similar complexities likely are common in other animal lineages as well. Reproducing organisms benefit from integrating all available information, from multiple modalities, and the resulting behaviours are unlikely to fit neatly into simple dichotomies or classification schemes as often used in behavioural research [22].

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