

Pheromones: Stink Fights in Lemurs

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Some primates, in particular lemurs, have long been known to communicate by smell. However, no bona fide primate pheromones have been identified. A recent study of ring-tailed lemurs offers some — disputed — candidate molecules for a male pheromone.

Many male mammals are notoriously smelly in the breeding season. This prompted Charles Darwin to suggest that these powerful smells were sexually selected signals as spectacular as a male peacock's tail [1]. Today we would call these 'male sex pheromones', evolved chemical signals within the species [2,3]. Pheromones have been identified in mammals of both sexes, for instance in mice and rabbits, as well as in a wide range of other animals from worms to fish [3]. However, despite evidence that smell is used for communication in primates, no primate pheromones have been chemically identified so far (despite claims for human pheromones [4]). A recent study in *Current Biology* by Mika Shirasu, Satomi Ito, Kazushige Touhara and colleagues [5] claims to have identified female-attracting odorants in male ring-tailed lemurs (*Lemur catta*). Their proposal has been questioned in this issue of *Current Biology* by Christine Drea and colleagues [6] and Peter Kappeler [7] — to which Shirasu, Ito and colleagues respond [8,9]. Their debate underlines the challenges of identifying pheromones in mammals.

Ring-tailed lemurs (Figure 1), like other strepsirrhine primates, are well known for their use of chemical communication in social behaviours [10]. These lemurs use multiple scent glands and sex-specific scent-marking repertoires. As well as genital scent glands, male ring-tailed lemurs have scent glands on their shoulders (brachial scent glands) which secrete a brown paste and wrist scent glands (antebrachial scent glands) which secrete small quantities of clear liquid [11]. Males sometimes mix the brachial gland and antebrachial gland secretions with a 'shoulder-rubbing' behaviour before 'wrist marking' the substrate with the antebrachial gland and spur with an audible click. Males also load their tails with secretions from these two glands and waft the smells towards opponents in characteristic 'stink fights'. The chemistry of the secretions produced by the genital glands of both sexes and the brachial glands of males has been investigated in depth [10, 11] but the volatile secretions of the male antebrachial glands and their possible function in male–female communication remained unknown.

Identifying pheromones in mammals is more challenging than in insects. First, mammals are really smelly, with hundreds of molecules revealed by gas chromatography. Each individual has its own chemical profile 'fingerprint' [3], influenced by factors including its genetics, sex, life stage, contributions from bacteria, recent diet and infections. Pheromones are superimposed on the highly variable individual chemical fingerprint. A male sex pheromone, for example, would be the same molecule or combination of molecules found in all mature males. The quantity of the pheromone

typically reflects the individual's hormonal state, itself influenced by season, nutritional state, and social status. Second, detecting and understanding mammal responses to pheromones is difficult. To identify a pheromone, a bioassay is needed, a repeatable behavioural or physiological response in the receiver [2]. For example, the wing-fluttering response of the male silk moth was used as the bioassay in Adolf Butenandt's pioneering 1950s work to identify the female silk moth sex pheromone [2]. Designing appropriate bioassays for mammals is hard as they typically do not have responses as stereotyped as insects (though insects' responses can vary too [2]). A further complication is that mammals may remember the individual odour fingerprints of conspecifics, indeed their social organization may depend on it. Bioassays of secretion samples thus need to control for familiarity: do responses differ to samples from familiar or unfamiliar individuals?

How do you find pheromones 'hidden' in a mammal's complex individual chemical profile? Darwin's observations suggest looking for molecules secreted more in the breeding than the non-breeding season. Drea and colleagues [11,12] had previously shown that lemur secretions and behaviours vary with season and reproductive state. In their recent study of male-specific antebrachial glands, Shirasu, Ito and colleagues [5] observed that females in their breeding season sniffed the gland secretions of breeding season males more than those of non-breeding season males. Three volatile molecules were found at higher levels in males' antebrachial glands during the breeding season: 12-methyltridecanal, tetradecanal and dodecanal. Compared to control odours, females sniffed longer at biologically relevant concentrations of two of the aldehydes (12-methyltridecanal and tetradecanal) and were attracted to a mixture of these plus the third aldehyde, dodecanal. Might the apparent seasonal difference in male gland secretion levels reflect breeding season testosterone titres and could this be experimentally manipulated? Shirasu, Ito and colleagues [5] injected two males in the non-breeding season with testosterone, which, they report, raised the candidate antebrachial gland molecules to breeding season levels.

The preliminary evidence for breeding season secretion of these particular molecules and seasonal female response led the authors [5] to cautiously propose that these molecules might constitute a male pheromone, while recognizing that the data rest on samples from only a handful of male lemurs and responses from small numbers of females, all in captive populations — which leads to the issues raised by Christine Drea and colleagues [6] and Peter Kappeler [7].

A key problem is the meaning of the sniffing bioassay in the biology of ring-tail lemurs. Like many studies of chemical communication in primates and other mammals, including some lemur studies by Drea and colleagues [12], Shirasu, Ito and colleagues [5] use the number and/or duration of sniffs to quantify the response by the receiving individuals. However, sniffing is not directly related to any particular behaviour or physiological response. For example, a longer sniffing response might simply indicate a response to novelty.

By contrast, rather than indirect measures such as sniffing, bioassays for sex pheromones in moths, fruit flies and mice have measured functional responses such as mating success or female readiness to mate [2,3]. Shirasu, Ito and colleagues [5] did not start with a phenomenon related to lemur mate choice or mating mediated by smell and build a bioassay from that. This would have been a challenge, but it is a relevant question because, apart from the ambiguity of the sniffing bioassay, it is not clear how the proposed secretions relate to the mating biology of ring-tailed lemurs. Drea and colleagues [6] and Kappeler [7] draw attention to the multi-male, multi-female group structure of ring-tailed lemurs and wonder about the role of the antebrachial secretions in mating biology. Both letters also ask how males would respond to the molecules.

Another problem, acknowledged by Shirasu, Ito and colleagues [5,8,9], is the small number of individuals: too much rests on two males. The number of females studied is larger but still small. The constraints are real: captive colonies are small, limiting both the number of males for sampling and females for responses. The chemistry, which has given definitive identifications more specific than previous studies [5,8], could not easily be done in the field. A further problem is the testosterone hormone manipulation. A longer term implantation of slow-release testosterone might have been better [6] and another control added [7]. However, animal welfare for captive primates precluded these [8,9]. As Shirasu, Ito and colleagues [5,8,9] agree, it is too early to say that these molecules constitute a pheromone. The importance of their study [5] is that it offers a starting point for further work; as they say, this preliminary study [5] needs to be repeated and extended with larger numbers of subject animals, as both secretion sources and responders, and with new bioassays, ideally also carried out in the wild.

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