Lack of Evidence for Pheromones in Lemurs

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eTOC summary

Drea *et al.* present arguments and evidence countering Shirasu *et al.*'s proposal of lemur pheromones. They challenge interpretations based on single-to-small samples, the discounting of animal learning and published data, unsuccessful endocrine manipulations, and over reliance on generalized sniffing and seasonal variation in scent composition.

As single or precisely ratioed chemicals that elicit unlearned, functionally specialized, and species-specific responses [1] or 'stereotyped behavior' [2], pheromones differ from mammalian scent signatures that comprise complex, variable mixtures, convey multiple messages via learned chemical combinations, and elicit generalized responses [1]. Studying ring-tailed lemur (*Lemur catta*) behavior and semiochemistry, Shirasu *et al.* [2] claim to have identified "the first sex pheromones in primates." Although we applaud their application of recent technologies to advance volatile compound identification, reliance on one male in most chemical procedures and on few females in behavioral procedures constrains statistical analyses and challenges the broad applicability of their findings. Also, the non-independent testing of even fewer signaler-recipient dyads downplays the critical role of learning and memory in primate communication [1] – an argument that refuted earlier claims of primate pheromones (reviewed in [3, 4]). Here, we challenge the authors' claim by addressing each of their four highlighted findings and interpretations.

First, Shirasu and colleagues noted that female lemurs were "more attracted to male scent gland secretion during the breeding season" (Figure 1 in [2]). That the scent of breeding (vs. nonbreeding) animals generates more sniffing has been long established (primates: [3]; lemurs: Table 1 in [4]); however, some of the bias, at least in *L. catta*, owes to the recipients' concurrent reproductive state (Figure 1A; [5]). Neglecting relevant behavioral studies [3-6] and constrained by sample limitations, the authors did not test other types of signaler-recipient dyads (i.e., female-female, male-female, male-male) or scents (i.e., labial, scrotal, brachial) necessary for contextualization (Figure 1B; [5]), nor did they control for signaler variables known to influence recipients, such as familiarity, dominance status, health, or genetic makeup [3-5]. For example, although *not* chemically encoded (note erroneous citation of Scordato *et al*. 2007 in [2]), the signaler's dominance status is nonetheless recognized in scent marks, owing to the lemurs' prior observation of behavioral interactions, retention of that social information, and later cross-modal integration with individual scent signatures [3, 5]. Most problematic, however, is the authors' interpretation that sniffing duration, which offers no functional socioecological insight, equates to pheromonal attraction. Such generalized investigation, necessary for olfactory processing, is not stereotyped, species- or sex-specific, uniquely directed to conspecific scent, or necessarily indicative of reproductive interest. Were that the case, one must conclude that female lemurs are most sexually attracted to *female* 'pheromones,' that change seasonally under hormonal control (Figure 1B; [7, 8]). The bioassay paradigm (using simultaneous, paired presentations of conspecific olfactory stimuli) tests only for discrimination, not for preference [9]. Stereotyped behavior (in this case, purported reproductive attraction) is thus not supported.

Second, Shirasu and colleagues claim that "three C12 and C14 aldehydes are seasonally secreted by the male antebrachial gland" and "are strong candidate compounds for female lemur attraction" [2]. That only one of four males (Bon) consistently met the seasonal criterion (Figure 2C in [2]) undermines this generalization. If such major variability were to exist in pheromone concentrations, it would suggest that only certain males could attract females and mate; however, this scenario is inconsistent with *L. catta* consortship. In this promiscuous, female-dominant species, females control reproduction [10] and sometimes mate with *all* candidate males during a single estrous cycle. Female choice may determine the order or frequency of partners, but mating is unlikely to be 'triggered' by male pheromones. Instead, condition-dependent olfactory signatures [4] likely influence which immigrant males can join the group. Seasonal differences in chemical composition, albeit confounded by other variables known to influence volatile compounds (e.g. diet, microbial communities; [1, 4]), thus suggest potential individual differences in signaler quality as a more parsimonious explanation. Contrary to claims of unavailable chemical data, primate researchers have identified seasonally varying and hormonally mediated chemical compounds, including ‘key’ semiochemicals (reviewed in [4]; [7, 8]), without labeling them pheromones. The Supplemental Information contains additional, new data from robust analyses of seasonal indicator compounds in male scrotal secretions; these preferred methods account for consistency across individuals. Shirasu and colleagues identified compounds that, at best, may contribute to such lists.

Third, the authors highlight as their most original and important contribution that “the amounts of the identified aldehydes increase in a testosterone-dependent manner” [2]. Unfortunately, their manipulation, attempted on only one male (Bon), failed to generate the purported endocrine effect. Whereas the authors featured Bon’s experimental testosterone values (in pink, Figure 3A in [2]), we highlight his second breeding-season value (in orange). Although illustrated on a discontinuous y axis, it is not an inconsequential outlier; rather, it uniquely represents peak breeding-season testosterone concentrations (Figure 1C; [10]). Instead of the appropriate within-subject comparison, the authors justify Bon’s experimental values by comparison to a breeding-season value from another, younger male (Doitsu). The suggested age difference, based on these two adults, is not borne out in a larger study [10], and the authors overlooked key findings that Bon’s experimental values remained largely within nonbreeding-season range [10]. Coupled with variability in their injection vs. sampling schedules (and the unknown time delay for steroidal action on odorants), the effect of treatment on Bon’s target compounds was minor, involving only one chemical. Producing a sustained increase in testosterone concentration might have been better served by using implants instead of injections. Had the manipulation worked, it would have joined the ranks of select endocrine manipulations achieved by chemical and surgical castration, hormonal contraception, or pregnancy [3, 4]. Nonetheless, the results would have provided additional evidence of endocrine modulation of *L. catta* odorants [8], not evidence of lemur pheromones.

Fourth, the authors highlight that “females are interested in cotton pads soaked in the identified aldehydes” [2], a statement that requires more nuance. Instead of testing odor-naïve females, as is recommended for any type of behavioral bioassay [9], reusing females tested earlier on conspecific scents ‘primed’ them for attending to these synthetic components. The authors also used sequential, go-no go procedures, in either single or group tests (Figure 4A and 4B, respectively, in [2]), despite that different procedures produce different patterns of discrimination and must be interpreted accordingly [9]. Contrary to the claim that these aldehydes “are key compounds…that elicit attractive behaviors …” [2], the authors merely showed that singly tested females *better detect* 12-methyltridecanal and tetradecanal at higher vs. lower concentrations. They also showed that in group tests, which are confounded by social facilitation, females were more ‘interested’ in *mixtures* than in isolated compounds, consistent with mammalian odor mosaics [1, 4] more so than with pheromonal action.

Were lemur pheromones to exist, their detection would require a different approach. For example, in reproductive contexts, female lemurs show a lordotic reflex, serving to realign their pelvis to allow intromission by the male. Unlike sniffing, lordosis is a sex-specific, reflexive (unlearned), and stereotyped response. Were male *L. catta* scent to induce lordosis in female *L. catta*, specifically, we would readily accept the existence of lemur pheromones. The study by Shirasu *et al.* [2], on seasonal and hormonal patterns in female behavior and male scent, replicates previous research on this species' chemical communication, but falls short of producing compelling evidence for the existence of primate pheromones.

SUPPLEMENTAL INFORMATION

Supplemental Information includes details on the statistical analyses and results (Tables S1 and S2) for Indicator Species Analyses, and can be found with this article online at xxx.

AUTHOR CONTRIBUTIONS

C.D. conceived of and wrote the correspondence, with input from J.C.C. and M.B., who also performed the chemical and statistical analyses presented in the Supplemental Material.

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FIGURE LEGEND

Figure 1. Seasonal variation in female behavioral responses to conspecific scent and in male and female androgen concentrations in ring-tailed lemurs (*Lemur catta*). (**A**) Female recipients in breeding condition (B_R, black) respond longer to scents from conspecific donors than do females in nonbreeding condition (N_R, white; * Ps < 0.05). (**B**) Females respond longer to labial scents than to male scrotal, brachial, or antebrachial scents; and to the combined scents from all donors in breeding (B_D, black) vs. nonbreeding (N_D, white) condition (Ps for main effects < 0.05). (**C**) Androgen concentrations, particularly testosterone in males, surge during the species' breeding season in the Northern hemisphere (double arrow). Modified from [5, 10].