

RESEARCH ARTICLE

Nasopalatine Ducts and Flehmen Behavior in the Mandrill: Reevaluating Olfactory Communication in Old World Primates

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Compared to other modes of communication, chemical signaling between conspecifics generally has been overlooked in Old World primates, despite the presence in this group of secretory glands and scent-marking behavior, as well as the confirmed production and perception of olfactory signals. In other mammalian species, flehmen is a behavior thought to transport primarily nonvolatile, aqueous-soluble odorants via specialized ducts to the vomeronasal organ (VNO). By contrast, Old World primates are traditionally thought to lack a functional VNO, relying instead on the main olfactory system to process volatile odorants from their environment. Here, in the mandrill (*Mandrillus sphinx*), we document unusual morphological and behavioral traits that typically are associated with the uptake of conspecific chemical cues for processing by an accessory olfactory system. Notably, we confirmed that both sexes possess open nasopalatine ducts and, in response to the presentation of conspecific odorants, we found that both sexes showed stereotyped behavior consistent with the flehmen response. If, as in other species, flehmen in the mandrill serves to mediate social or reproductive information, we expected its occurrence to vary with characteristics of either the signaler or receiver. Flehmen, particularly in a given male, occurred most often in response to odorants derived from male, as opposed to female, conspecifics. Moreover, odorants derived during the breeding season elicited more flehmen responses than did odorants collected during the birthing season. Lastly, odorants from reproductively cycling females also elicited more responses than did odorants from contracepted females. Although confirming a link between the nasopalatine ducts, flehmen behavior, and olfactory processing in mandrills would require further study, our observations provide new information to suggest anatomical variability within Old World primates, calling further attention to the underappreciated role of chemical communication in this lineage. *Am. J. Primatol.* 9999:1–12, 2013. © 2013 Wiley Periodicals, Inc.

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INTRODUCTION

Relative to the visual sense, the olfactory sense is underappreciated in primates [Heymann, 2006], particularly as a means of intraspecific communication—this, despite the prominence of olfactory communication among other vertebrates [Müller-Schwarze, 2006]. Within the order Primate, chemical communication is least well understood in Old World species. Our general lack of understanding can be attributed to a long-held view that elaboration of the visual system, characteristic of Old World species, came at the expense of olfactory specialization; hence, olfactory capabilities in this lineage were thought to have been diminished or even lost over evolutionary time [Barton, 1998, 2006; Heymann, 2006]. This view is challenged, however, by a growing body of work on humans, in such areas as olfactory-guided mate choice [Jacob et al., 2002; Milinski & Wedekind, 2001; Ober, 1999; Wedekind & Furi, 1997] and odor-based individual recognition [Schaal et al., 2006; Schaal &

Durand, 2012]. To bridge the “olfactory gap” between New World monkeys and humans, we have launched a multipronged (i.e., gross morphological, chemical, observational, and experimental) study of olfactory communication in the mandrill (*Mandrillus sphinx*). As noted by Darwin, “no other member in the whole

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class of mammals is coloured in so extraordinary a manner as the adult male mandrill” [Darwin, 1871]. According to the traditional view, this species’ exceptional reliance on visual cues for intraspecific communication [Renoult et al., 2011; Setchell & Wickings, 2005] would make it an unlikely candidate for reliance on olfactory signals. Nevertheless, we present two lines of evidence in the mandrill that together prompt a re-evaluation of the assumptions about olfactory communication in Old World species.

Most mammals possess two distinct sets of chemosensory neurons located, respectively, in the main olfactory epithelium (MOE) and in the vomeronasal organ (VNO). Both structures are neurally segregated and are generally thought to function in processing different kinds of information [Berghard et al., 1996; Halpern & Martínez-Marcos, 2003]. Notably, the MOE is specialized in sensing small, volatile chemicals, derived from numerous environmental sources, whereas the VNO is specialized in sensing fluid-phase molecules, particularly nonvolatile odorants emitted by conspecifics that are thought to mediate reproductive activity [Del Punta et al., 2002; Kimchi et al., 2007]. Despite clear morphological differences between the MOE and the VNO, comparable functions increasingly are being attributed to both systems, thereby blurring the functional distinction between them. In rodents, for instance, both the MOE and the VNO respond to pheromones by inducing similar behavioral and endocrine changes [Boehm et al., 2005; Dulac & Wagner, 2006; Leybold et al., 2002; Lin et al., 2005; Yoon et al., 2005]. Nonetheless, there has been much debate over the varying role of the VNO in primate olfactory communication [Evans, 2006; Meredith, 2001; Smith et al., 2001a].

Notably, whereas strepsirrhines and New World monkeys are generally considered to possess a functional VNO containing micro-anatomical features (i.e., vomeronasal neurons) [Aujard, 1997; Schilling et al., 1990; Smith et al., 2011], albeit with substantial variation between species [Smith et al., 2011], Old World primates are generally considered to lack a functional, primitive mammalian VNO [Ankel-Simons, 2000; Frets, 1914; Jordan, 1972; Loo, 1973; Maier, 1997]. Certain Old World primates (e.g., humans, macaques) possess an embryonic structure with bipolar neurons that resembles a VNO [Boehm & Gasser, 1993; Wilson & Hendrickx, 1977]; however, this VNO-like structure remains vestigial in adulthood [Ankel-Simons, 2000; Hunter et al., 1984; Maier, 1997]. Although some adult humans seem to possess a VNO-like structure [Trotier et al., 2000; Won et al., 2000], the cells present in this modified VNO that resemble sensory neurons are not known to connect to the brain ([Trotier et al., 2000] and see Box 1 in [Dulac & Torello, 2003]). Because VNO anatomy is variable

across the few Old World species studied [Smith et al., 2001a, 2002], it is possible that further anatomical studies, particularly of the region surrounding the septal–palatal junction, could reveal the presence of an atypical VNO in certain catarrhines. In other words, the purported absence of a VNO in this lineage might reflect the scarcity of relevant, systematic studies [Smith et al., 2001a]. Certainly, the impact of structural variability on VNO functionality remains to be explored.

Depending on the species, the VNO can open into the nasal cavity via vomeronasal ducts or into the mouth via nasopalatine or incisive ducts [Hunter et al., 1984; Smith et al., 2001a]. If the incisive duct is present and open at both ends, the VNO could gain access to stimuli via either the nasal or buccal route. Several mammalian groups (including lagomorphs, ungulates, and carnivorans: reviewed in [Estes, 1972; Schneider, 1930]) purportedly use specialized behavior, namely the stereotypical flehmen response, to transport nonvolatile chemosignals into the lumen of the VNO. Transport generally occurs through the oral cavity and nasopalatine ducts [Doving & Trotier, 1998; Halpern, 1987; Johnston, 1998] using an active vascular pumping mechanism to expose receptors of the accessory sensory neurons to the nonvolatile odorants [Meredith, 1994; Meredith et al., 1980]. The behavioral sequence of flehmen varies among species, but the animal typically lifts its head after investigating an odorant source (such as urine) either by sniffing or licking, then wrinkles its nose, and lifts its upper lip in a characteristic “grin.” Although researchers have established a connection between flehmen and the VNO in the goat using tracer dyes [Ladewig & Hart, 1980; Melese-d’Hospital & Hart, 1985], much of the evidence-linking flehmen to a functional VNO is indirect. Among antelopes, for instance, the two species (topi and Coke’s hartebeest) that lack incisive papilla and nasopalatine ducts are those that fail to show flehmen. Compared to males of other antelope species, the males of these two species also show reduced sexual arousal when exposed to conspecific female urine [Hart et al., 1988]. Researchers also have relied on indirect evidence to suggest alternate VNO pathways. For instance, in another antelope species (wildebeest) that lacks incisive papilla, but has small incisive ducts, males perform a modified form of flehmen, which involves licking the odorant source and then licking their nostrils [Hart et al., 1988]. Given that oral access to the VNO is impaired, this modified behavior could deliver the odorant to the VNO via a nasal route [Hart et al., 1988]. To date, we lack any comparable information about flehmen or associated behavior in any Old World primate.

Beyond the anatomical evidence, there is also genetic evidence to support the evolutionary loss of olfactory capabilities in Old World primates. For instance, most genes coding for pheromone receptors in rodents, such as two families of G-protein-coupled

receptors, are non-functional pseudogenes in humans [Dulac & Axel, 1995; Kouros-Mehr et al., 2001; Pantages & Dulac, 2000]; however, they are also pseudogenes in marmosets, a New World primate characterized by a functional VNO [Giorgi & Rouquier, 2002]. Moreover, the gene coding for the ion channel tyrosinase-related protein 2 (TRP2), which plays a major role in VNO function in mice [Stowers et al., 2002], is also a pseudogene in humans [Liman et al., 1999]. Additionally, there is an absence of selective pressure on TRP2 function in Old World primates [Liman & Innan, 2003; Zhang & Webb, 2003]. Some authors have even suggested that pheromone communication has been lost in this lineage and replaced by trichromatic vision, which emerged at a comparable time (~23 MYA) in the evolutionary record [Liman & Innan, 2003; Zhang & Webb, 2003]. Perhaps the conspicuous red, sexual swellings of females in several Old World species constitute observable signals that substituted for pheromones [Zhang & Webb, 2003]. Other evidence, however, is incompatible with the purported trade-off between olfactory capabilities and trichromatic vision [Matsui et al., 2010], including with regard to the perception of sexual signals [Curtis et al., 1971; Leonhardt et al., 2009]. In sum, species may possess different functional vomeronasal genes [Giorgi & Rouquier, 2002] and Old World primates may not be as microsmatic as previously thought [Adipietro et al., 2012; Hübener & Laska, 1998; Laska et al., 2005].

The presumed absence of any significant chemical communication in Old World primates is additionally challenged by the existence of specialized scent glands (e.g., gibbon: [Geissman, 1987]; human: [Schaal et al., 2006] and see for a review: [Dixson, 1998]), the display of scent-marking behavior (e.g., vervet monkey: [Freeman et al., 2012]; guenon: [de Jong & Butynski, 2010; Loireau & Gautier-Hion, 1988]), the production of known semiochemicals (e.g., macaque: [Curtis et al., 1971]; human: [Penn et al., 2007]), and the perception of these cues by conspecifics (e.g., macaque: [Laska et al., 2005; Michael & Keverne, 1968]; human: [Wedekind & Furi, 1997]). In no case, however, have these complementary lines of evidence been integrated within a single species.

Among Old World primates, the mandrill is an ideal candidate model for a multipronged study of intraspecific chemical communication. Mandrills occur in multimale–multifemale groups, with moderately seasonal breeding [Setchell & Wickings, 2004]. They are male dominant [Dixson et al., 1993], extremely sexually dimorphic [Setchell & Dixson, 2001], and highly polygynous, with alpha males securing about 70% of conceptions [Charpentier et al., 2005]. Relative to most anthropoids, mandrills of both sexes are distinguished by possession of a secretory, sternal gland [Hill, 1970] (Fig. 1A).

All group members scent mark, but males invariably mark more than do females and dominant individuals mark more than do subordinates [Feistner, 1991]. Researchers also have found patterns in the chemical secretions of mandrills that may underlie the communication of socially relevant information [Setchell et al., 2010, 2011]. Here, we extend these studies by confirming Hill's [1970] notation about a morphological feature of the mandrill palate and providing new information about the behavioral response of male and female mandrills to the controlled presentation of conspecific odorants. Given the suite of morphological, behavioral, and chemical specializations, we suggest that olfaction plays a more major role in the communication system of mandrills than previously anticipated.

METHODS

The mandrills were housed in accordance with the Comité Régional d'Éthique pour l'Expérimentation Animale, Ile-de-France Sud, and our protocols were approved by Duke University's Institutional Animal Care and Use Committee (Protocol Registry Number A192-07-07), as well as by the Gabonese authorities (CENAREST Permit Number: AR0006/08). Our research adhered to the legal requirements of Gabon and to the American Society of Primatologists' principles for the ethical treatment of nonhuman primates.

Subjects and Housing

Our subjects derived from the primarily semi-free-ranging mandrill colony at the Centre International de Recherches Médicales de Franceville (CIRMF), in Gabon. This colony was established in 1983–1984 with 15 unrelated, wild founders [Wickings, 1995] and by 2008, at the start of our study on mandrill olfactory communication, numbered around 200 individuals. Our focal subjects for behavioral testing (i.e., the odorant “recipients”) were 21 adults (9 males, 12 females). At the onset of the bioassays (in 2009), the males were aged 6.7–21.2 years and the females were aged 6.3–37.3 years. The males were reproductively intact, whereas all but one of the females (a post-reproductive female) were hormonally contracepted with etonogestrel (Implanon®). Additionally, 116 mandrills (50 males aged 2.6–21.1 and 66 females aged 1.7–36.4 at the onset of the bioassays) served as odorant “donors” (see below). The female donors represented both contracepted and reproductively cycling animals.

The majority of the CIRMF mandrills live in one of three large, social groups inhabiting rainforest enclosures (0.5–6 ha, hereafter referred to as the “forest population.” Each forest enclosure has an adjoining capture area (~5 m × 5 m), comprising two separable sections in which animals can be

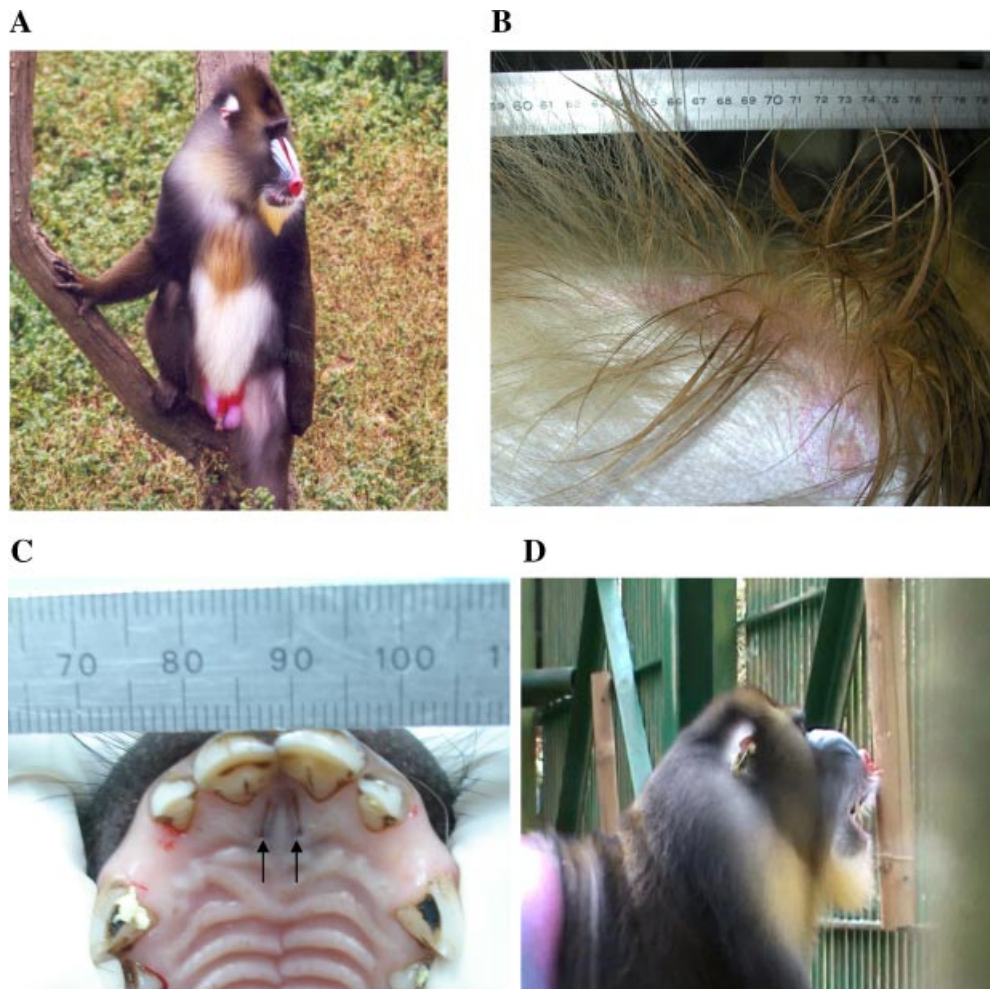


Fig. 1. Morphological and behavioral traits associated with olfactory communication in the mandrill. Shown are (A) an adult male, with long golden, chest hair denoting the location of the sternal gland; (B) a close-up of the sternal gland (triangular, pink area) in a supine alpha male, showing chest hair imbued with greasy, glandular secretions; (C) the palate of an adult female, showing the paired entrances of nasopalatine or incisive ducts (see indicative arrows) located either side of the nasopalatine papilla, caudally to the upper incisors; and (D) an adult male performing an open-mouth or flehmen behavior in response to conspecific odorant presented on a bamboo pole.

temporarily isolated. The remaining CIRMF mandrills ($N = 17$) live in one of four social units, each inhabiting small ($\sim 7 \text{ m} \times 7 \text{ m}$), outdoor pens, hereafter referred to as the “pen population.” These pens can be partitioned, also to facilitate temporary isolation of subjects. Our focal subjects included seven animals from the forest population and 14 animals from the pen population. All of the mandrills had freely available water and were provisioned twice daily with a noncommercial monkey diet (a molasses and flour-based cake that is prepared on site) and locally available fruit. The mandrills in the forest population supplement this diet with food foraged from the rainforest.

The mandrills have been observed routinely since the establishment of the colony, so that the dominance status of group members can be determined or verified based on approach-avoidance behavior. Young animals and adult females acquire their maternal dominance rank. Thus, all females

from a given matriline possess relatively comparable ranks, generally with the matriarch being the highest-ranking member and the youngest daughter ranking above older daughters. Moreover, within a social group, the different matriline are linearly ranked, such that the females within a matriline all rank above (or all rank below) the females within another matriline [Setchell, 1999]. Female rank is thus stable over time. By contrast, dominance rank in adult males depends on individual competitive abilities and is therefore contested and transient. The alpha male could be readily identified by his ability to displace all of the other males in the group (or more routinely, by their avoidance of him) and by his more prominent secondary sexual characteristics. Attributing a specific rank (i.e., other than merely “subordinate”) to the few adult males at the bottom of the hierarchy can be challenging, as these may not be permanent members of the group and rarely interact with resident members [Setchell et al., 2006]. We

verified the social status of our subjects at the time of odorant sampling and behavioral testing. For our purposes in the present study, given the limited sample size of focal subjects, we used only two categories of social status per sex. We discriminated between the alpha male (dominant) versus all other adult males (subordinate), and between adult females in the top-ranked matriline of each study group (dominant) versus adult females from all the other matriline (subordinate). Based on these criteria, our focal subjects included two dominant males, seven subordinate males, five dominant females, and seven subordinate females. Likewise, our odorant donors included five dominant males, 45 subordinate males, 21 dominant females, and 45 subordinate females.

Odorant Sample Collection and Photographic Records

The mandrills are captured annually at the CIRMF for routine physical examinations, for which they are anesthetized by intramuscular injections of ketamine (Imalgène 1000; 10 mg/kg body weight) administered via blowpipe by the veterinary personnel. During physical examinations spanning 2008–2010, we collected odorant samples from the different scent “sources” (i.e., sternal, scrotal/vaginal, and salivary) of the 116 donors. Following published procedures [Scordato & Drea, 2007], we rubbed pre-cleaned cotton swabs, held with clean forceps, repeatedly against the sternal gland (in both sexes; the male sternal gland is shown in Fig. 1B), against the genitals (scrotum in males; vagina in females), or inside the cheek (to collect saliva from both sexes). We stored samples at -80°C until their use in behavioral bioassays (described below). During the course of the study, we obtained odorant samples year round, during periods representing the birth season (November–March) and the breeding season (April–October). During some of the physical examinations, while the animals were in hand, we opportunistically photographed the palate of representative males and females (Fig. 1C).

Behavioral Bioassays

Our behavioral bioassays followed published procedures [Scordato & Drea, 2007] and were aimed at testing the mandrills’ ability to discriminate between the individual traits of conspecifics based on odor alone. We will detail those findings elsewhere. Here, we report on a subset of the recipients’ behavioral responses in regard to characteristics of the odorant donor, including sex, age, and dominance status. We performed 282 behavioral bioassays, from May 2009 to July 2010 (comprising the two seasons defined above), on our focal mandrills (1–37 tests per recipient; mean number of tests per recipient: $13.4 \pm \text{SD } 10.6$). We always presented recipients

with two matched odorants (i.e., originating from the same type of scent source) from two unfamiliar donors, as well as with an unscented “control” sample. We defined animals as being “unfamiliar” if they had never lived in the same social group; although, in most cases, the donors were actually unknown to the recipients, having never even shared a fence boundary. Collapsing across odorant type, the donors served for 1–17 behavioral bioassays each (mean number of tests per donor: $4.8 \pm 4.0 \text{ SD}$).

Under each housing condition (see above), both a “holding” arena and a “test” arena were available. The test arenas were comparable in size across housing conditions. We could thus isolate the recipient into the holding arena while we prepared the test arena for the bioassay. We attached three new and freshly scented bamboo shoots ($\sim 50 \text{ cm}$ in length $\times 5 \text{ cm}$ in diameter) to the inside of the fencing. We placed them vertically at heights appropriate to the recipient’s sex (i.e., at about 20 or 50 cm above the ground for females and males, respectively) and positioned the three shoots 1 m apart, along one side of the test arena, opposite the doorway connecting the two arenas (Fig. 1D). During an initial period of habituation, we rubbed the shoots with various commercially available, edible odorants (e.g., extracts of almond, banana, orange, etc.). Once the mandrills were familiar with the procedures and readily investigated the bamboo, we began testing. We rubbed each outer “test” shoot with one of two donors’ secretions, respectively, and rubbed the center “control” shoot with a clean cotton swab. We then released the recipient into the test arena and began recording behavior. We video recorded the behavioral response to the three shoots during 15-min trials. Two observers scored the trials and recorded all occurrences of olfactory behavior, including sniffing, licking, marking with the sternal gland, and an open-mouth response, hereafter called “flehmen” (Fig. 1D). Flehmen differed markedly from yawning—a common display behavior in mandrills [Baenninger, 1987]—in that the lips were not retracted to expose the canines. The observers also scored other behavior that we collapsed into a category called “interest,” which included approaching within 1 m of the shoot in association with any of the following: staring at, biting, touching, or snatching the shoot.

Analyses

For the occurrences of flehmen observed in our focal subjects (see Results Section), we first tested for any influence of the recipients’ characteristics: For recipient sex and dominance status, we used Fisher’s exact test, and for recipient age at the beginning of the study, we used Mann–Whitney’s *U*-test. Next, we tested for any influence of the season in which the tests were performed, using Fisher’s exact test. Given the flehmen patterns observed in other species, we expected more males than females to perform this

behavior, especially during the reproductive season. Lastly, for the recipients displaying flehmen (see Results Section), we tested if the number of flehmen occurrences was correlated to the number of bioassays performed, using Spearman's rank correlation test.

Both the small sample size of recipients displaying flehmen and their highly skewed responsiveness limited formal analyses of the influence of the odorant donors' characteristics. Therefore, we pooled both sexes of recipients and performed descriptive analyses of their responses restricting the data set to the 134 tests performed by the five male and female recipients that displayed this behavior. We considered the donor's sex, dominance status, and age at first odorant collection. We further examined the occurrence of flehmen for any influence of (a) the season in which the odorants were collected (i.e., breeding or birthing), (b) the female donor's contraceptive status (i.e., contracepted or not contracepted), and (c) the odorant type (i.e., sternal, scrotal/vaginal, or salivary). Because the subset of 134 tests (see above) involved 82 odorant donors that were sampled under different seasonal or contraceptive conditions and from different scent sources, our calculations involved 268 different samples (including 132 female-only samples for the calculations involving contraceptive status). In this set of summations, we avoided pseudo-replication by considering the absolute occurrence (yes or no) of flehmen responses by a recipient toward a given odorant, as opposed to the total frequency of its flehmen responses toward that odorant. Again because of patterns observed in other species, we expected that the vaginal odorants from cycling females would elicit the most flehmen responses.

Lastly, to gain insight into the potential role of flehmen in olfactory and/or gustatory communication, we examined if specific behavior may have immediately preceded or followed flehmen. Notably, if flehmen plays a role in olfaction, one might expect it to be closely associated with sniffing, whereas if flehmen plays a role in gustation, one might expect it to be closely associated with licking. We calculated the probability of observing each type of behavior prior to or after flehmen from the overall frequency with which that

behavior was observed during all trials involving the five recipients that performed flehmen. We then performed Binomial tests on these observed and expected frequencies. We performed all tests, which were two-tailed, using R statistical software and considered P values ≤ 0.05 to be significant.

RESULTS

Anatomical Observations

The sternal glands of adult animals were clearly secretory, producing a thick, greasy substance that accumulated on the skin surface and surrounding hair, and were most pronounced in dominant males (Fig. 1B). All of the animals examined (including juveniles, subadults, and adults of both sexes) possessed a pair of nasopalatine or incisive ducts, each aperture measuring ~ 1 cm in length, located anterior to the first palatal ridge (Fig. 1C). The two nasopalatine papillae were separated by ~ 3 mm, which corresponds to the distance observed in platyrrhines; however, the shape of the ducts was more similar to that observed in *Aotus* than to that observed in *Callithrix* or *Ateles* (see Fig. 1 in [Hunter et al., 1984]).

Observations of Flehmen Behavior

From a total of 282 trials presenting conspecific odorants to 21 adult, recipient mandrills, which represented slightly over 70 h of behavioral observation, we recorded 69 events of flehmen (Fig. 1D). Therefore, while in the proximity of conspecific olfactory stimuli, mandrills on average performed roughly one flehmen per hour. In this species, flehmen involved a wide-open mouth held in front of, and in proximity to, an odoriferous substrate, sometimes accompanied by a distinctive oral aspiration (see also supplementary video).

Characteristics of the Recipients Performing Flehmen

Of the 21 focal subjects (i.e., the recipients), 5 (23.8%) showed the flehmen response (Table I). Flehmen was displayed by one-third of the males

TABLE I. Characteristics of the Recipients That Displayed Flehmen Behavior During Bioassays

Animal ID	Recipient characteristics			No. of bioassays received	Total flehmen responses given
	Sex	Social status	Age (year)		
2H	Male	Subordinate	18.3	37	1
10i	Male	Dominant	14.9	33	64
27	Male	Subordinate	$\sim 8.1^a$	28	1
28	Female	Dominant	$\sim 10.8^a$	19	1
5N	Female	Subordinate	8.2	17	2

Note: Ages are reported for the beginning of the bioassay study in 2009.

^aAges were estimated for animals that originated in the wild.

tested and one-sixth of the females tested. As these ratios were not significantly different from one another (Fisher exact test: odds ratio = 2.39, $P = 0.61$), recipient sex did not predict the occurrence of flehmen. The distribution of occurrences among these five individuals was highly skewed, however, as one male displayed a majority (64 or 92.7%) of the recorded events. This male's behavior could not be explained by the number of bioassays conducted, as there was no relation between the number of tests performed by any individual and the number of flehmen responses displayed by that individual (Spearman correlation test: $N = 21$, $r_s = -0.11$, $P = 0.86$; Table I).

The dominance status of recipients also did not predict the occurrence of flehmen, as 2/7 (28.6%) dominant animals and 3/14 (21.4%) subordinate animals displayed flehmen (Fisher's exact test: odds ratio = 0.69, $P = 1$). Likewise, recipient age did not predict the occurrence of flehmen: The average age of adult individuals displaying flehmen ($12.2 \pm \text{SD } 4.4$ years) was the same as that of adult individuals not displaying flehmen ($14.6 \pm \text{SD } 7.9$ years; Mann-Whitney's U -test: $W = 38$, $P = 0.90$). Lastly, although flehmen was observed in only 3/53 (5.7%) trials during the birth season, but in 28/229 (12.2%) trials during the breeding season, the season in which the bioassays were performed also did not reliably influence the occurrence of flehmen (Fisher's exact test: odds ratio = 2.32, $P = 0.22$); however, this latter result may be biased by the one male recipient that contributed disproportionately to the data set.

Characteristics of the Donors Whose Odorants Induced Flehmen

Of the 116 donors, 27 (23.2%) had their odorants inspected by flehmen. Flehmen was elicited by odorants derived from animals of both sexes, of each dominance status, and of all different adult ages. Moreover, odorants from all four scent sources elicited flehmen behavior. In addition, recipients directed 17 (24.6%) flehmen responses towards the unscented or control bamboo shoot. In only three trials, however, was flehmen directed solely to a control shoot; in all other cases when flehmen was directed to a control shoot, the recipient also had directed flehmen to a scented shoot during the same trial.

For the following descriptive statistics, we discarded the 17 flehmen responses to control bamboo and restricted our calculations to all the behavioral bioassays performed by only those five recipients that displayed flehmen ($N = 134$ trials involving $N = 82$ odorant donors). In these cases, male scent received more than twice as many flehmen responses as did female scent (47.1% of males vs. 22.9% of females received a flehmen response; Fig. 2A). We did not observe any obvious influence of the dominance

status of the odorant donor, as 33.3% of dominant individuals versus 32.8% of subordinates elicited a flehmen response (Fig. 2A). Likewise, the average age of donors whose odorants elicited flehmen was comparable to the average age of donors whose odorants did not elicit flehmen ($9.2 \pm \text{SD } 4.0$ years and $9.8 \pm \text{SD } 6.9$ years, respectively).

When considering the broad reproductive state of donors, we found that odorants collected during the breeding season elicited more than twice as many occurrences of flehmen (18.9%) as did odorants collected during the birthing season (8.8%; Fig. 2B). Also, the female donor's contraceptive status may have influenced the occurrence of flehmen by the recipients, as 14.8% of cycling females compared to 8% of contracepted females elicited flehmen (Fig. 2B).

Lastly, of the 268 odorant samples presented to mandrills, 192 samples were derived from the sternal area, of which 31 (16.1%) elicited flehmen, 10 derived from the testicular area, of which two (20%) elicited flehmen, 46 derived from the vaginal area, of which three (6.5%) elicited flehmen, and 20 derived from saliva, of which one (5%) elicited flehmen.

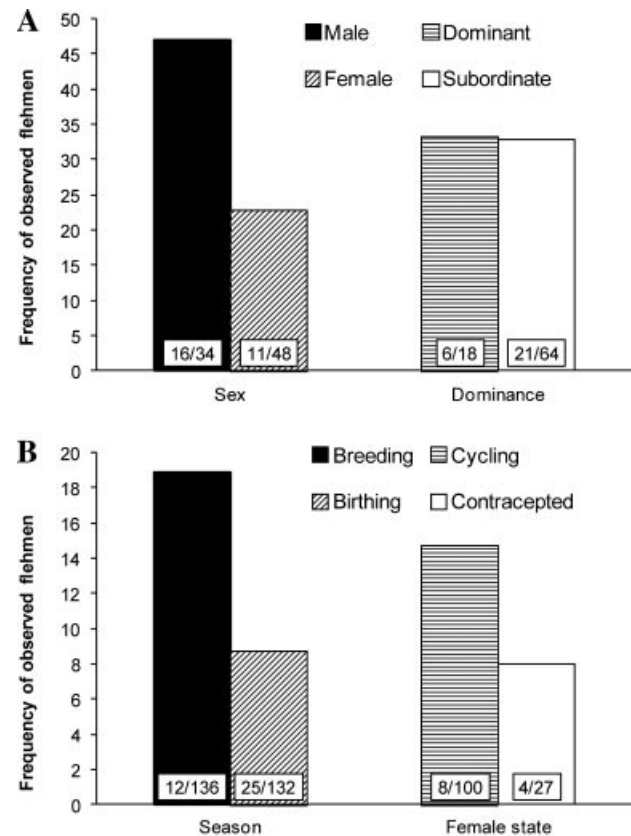


Fig. 2. Frequency of the odorants presented that elicited a flehmen response as a function of the donor's (A) demographic characteristics and (B) reproductive parameters. The numbers at the bottom of each bar represent the total flehmen responses observed, relative to the total number of trials presenting odorants for the variable considered.

Differences in the Behavior Flanking Flehmen

In the five individuals that displayed flehmen, we looked for patterns in the behavior preceding and following flehmen (Table II). We found that both sniffing and scent marking preceded flehmen significantly more often than expected by chance, whereas other behavior that did not specifically involve olfaction, such as interest (as defined in methods), was observed to precede flehmen significantly less often than expected by chance. Most notably, licking did not occur prior to or following flehmen any more frequently than expected.

DISCUSSION

Mandrills have long been renowned for their striking visual signals or secondary sexual adornments [Darwin, 1871], but they are far less well known for their intraspecific olfactory repertoire. Here, we provide new information in the mandrill about the presence of specific morphological and behavioral traits that are typically associated with olfactory communication. Notably, consistent with Hill [1970], we found an open pair of nasopalatine ducts in animals of all ages and both sexes. In addition, one quarter of the adult mandrills tested, including animals of both sexes, showed an open-mouth response to the presentation of conspecific odorants. Nonetheless, one male accounted for the majority of responses; were it not for this male, flehmen in mandrills would be a relatively rare event. To the extent that his preferences were representative, then contrary to our expectation, flehmen in mandrills might occur more often in response to male odorants. In keeping with our prediction, however, flehmen in mandrills might occur most often to conspecific odorants expressed during the breeding season. This first set of results is consistent with a role for flehmen in monitoring sex or reproductive state. Moreover, as expected, reproductively cycling females might elicit more flehmen responses than would contracepted (or noncycling) females. Lastly,

the type of odorant could also influence the occurrence of flehmen. Although these results are preliminary, they draw attention to an unusual behavior in an Old World primate that merits further study.

At present, any suggestion of functional significance of the mandrill's flehmen response is speculative, as confirmation of functionality would also require further study. Nonetheless, combined with the presence of a secretory sternal gland [Hill, 1970] (Fig. 1A,B), well-defined scent-marking behavior [Feistner, 1991], and the production of known semi-chemicals [Setchell et al., 2010], both the presence of incisive ducts and the display of flehmen behavior suggest either that mandrills are unusually reliant on chemical signals relative to other members of their lineage or that the role of chemical communication in Old World primates, more generally, has been underestimated.

With regard to Old World primates, the most widely held view is that the VNO is either absent or vestigial and nonfunctional [Ankel-Simons, 2000; Frets, 1914; Jordan, 1972; Loo, 1973; Maier, 1997]. Indeed, a primitive mammalian VNO is clearly absent in adults of almost all primate species studied, including baboons, various macaques and one colobine species [Smith et al., 2001a], but there is evidence that adult humans and chimpanzees have retained a VNO-like structure [Bhatnagar & Smith, 2001; Johnson et al., 1985; Smith et al., 1998, 2001b], the function of which remains unclear [Meredith, 2001]. In most other species, however, little is known about the presence of VNO-like structures or even about the presence of incisive ducts. Although some species, such as the mangabey [Hill, 1974], may possess naso-palatine papillae and adjacent duct apertures that have a complex macroscopic appearance, our understanding is further limited by the fact that the presence of these structures need not reflect a functional accessory olfactory system (AOS). For instance, in humans, a degeneration of vomeronasal nerves has been recorded after 23 weeks of gestation [Boehm & Gasser, 1993; Boehm et al., 1994]; a similar

TABLE II. Comparison of the Observed Versus Expected Behavior Occurring Prior to and After Each Flehmen Response

Behavior	No. of times preceding flehmen			No. of times following flehmen		
	Observed	Expected ^a	<i>P</i> -value ^b	Observed	Expected ^a	<i>P</i> -value ^b
Interest	16	38	<0.0001	9	19	<0.001
Flehmen	2	2	1	2	1	0.28
Licking	3	5	0.63	4	2	0.30
Marking	6	1	<0.0001	0	0	1
Sniffing	41	23	<0.0001	19	11	<0.01

^aRounded to the nearest integer.

^bBinomial tests.

degeneration is also possible in macaques and baboons [Hendrickx, 1971; Wilson & Hendrickx, 1977]. Moreover, whereas rhesus macaques (*Macaca mulatta*) also possess incisive ducts, they lack a VNO [Zingesser, 1984]. Thus, the presence of patent nasopalatine ducts in the mandrill is insufficient evidence to suggest a functional VNO in this species.

Consistent with the anatomical evidence, to our knowledge, flehmen behavior has not been previously reported in any Old World primate. Although flehmen and other associated behavior (such as lip smacking or tongue protrusion) are recognized in strepsirrhines and New World monkeys, and may serve a comparable function [Epple, 1974; Jolly, 1966; Wolowich & Evans, 2007], the complete behavioral sequence of the flehmen response has been reported only for the ring-tailed lemur (*Lemur catta*) [Bailey, 1978]. Contrary to prior studies [Dugmore et al., 1984], however, we have not seen any evidence of flehmen in our own olfactory studies of *L. catta*, despite having observed frequent licking of conspecific odorants [Charpentier et al., 2010; Crawford et al., 2011; Drea & Scordato, 2007; Scordato & Drea, 2007]. Moreover, using comparable testing conditions across species, we note that, even if we were to exclude the most responsive male mandrill, our mandrill subjects showed flehmen more frequently than did our lemur subjects (but see [Dugmore et al., 1984]). Although it is possible that this behavior serves no function in the mandrill, flehmen is typically thought to be associated with the transport of odorants to the VNO. Of particular relevance is that the other primate species reported to perform flehmen [see Hunter et al., 1984] also present a functional VNO [Evans & Schilling, 1995; Hunter et al., 1984; Mendoza et al., 1994], coupled with secretory scent glands and scent-marking behavior [Epple et al., 1993; Schilling, 1979]. Nonetheless, in horses, which are reputed for their characteristic flehmen response, incisive ducts are present, but are blind ending and thus do not communicate with the VNO [Lindsay & Burton, 1983; Salazar et al., 1997]. Thus, further experimental or anatomical studies are needed to confirm that flehmen in the mandrill serves to transport odorants via the nasopalatine ducts to a VNO or related structure.

With regard to the mandrills' behavioral patterns, we found no sex difference in the display of flehmen, even though most of the female recipients were contracepted. Although in various species both sexes are known to perform flehmen, including to environmental or predator odorants [Crowell-Davis & Haupt, 1985], flehmen in response to conspecific odorants is typically more frequent in males, especially in response to odorants from reproductive females (e.g., bulls: [Sankar & Archunan, 2002]; elephants: [Rasmussen, 1998]; cats: [Hart & Leedy, 1987]). Based on the typical pattern found

in other species, we might have expected vaginal odorants from reproductively intact, female mandrills to produce the greatest flehmen response. As expected, reproductive females seemed to elicit more flehmen responses than contracepted females, and samples collected during the breeding season also seemed to elicit more flehmen responses than samples collected during the birthing season. Contrary to expectations, however, male odorants elicited twice as many flehmen responses as did female odorants. Given the small sample sizes contributing to these patterns, as well as the disproportionate responsiveness of one male, these findings should be interpreted with caution. Nonetheless, these data provide some evidence to suggest that flehmen in mandrills may be influenced by the sex or reproductive state of the odorant donor.

Given the anatomical variability and debate surrounding the catarrhine VNO, it may be necessary to consider functional plasticity in the flehmen response. For instance, flehmen may serve to link the olfactory and gustatory senses. Several mammalian species possess taste buds located in the epithelium of the palatine papillae [Borcherding & Wöhrmann-Repenning, 2007; Hofer, 1977; Schmidt & Wöhrmann-Repenning, 2004; Wöhrmann-Repenning, 1978], the presence, specific location, and function of which are highly variable across species [Schmidt & Wöhrmann-Repenning, 2004]. Assuming the presence of receptor organs such as taste buds, flehmen in the mandrill may facilitate gustatory processing of chemical signals. Presumably, gustatory investigation of nonvolatile, aqueous-soluble molecules through direct physical contact facilitates odorant transport to the VNO; however, the view that the VNO is involved only in the detection of nonvolatile molecules is challenged by the fact that VNO neurons can express sensitive and specific responses to volatile compounds from both urinary and body odorants [Keller et al., 2009; Xu et al., 2005]. Accordingly, depending on species-specific pathways in the uptake and transport of odorants, licking may not be necessary to elicit flehmen. Were flehmen in the mandrill to facilitate transport of molecules to a VNO-like structure or to the MOE, these molecules would likely include volatiles, given that sniffing, but not licking, occurred more often in association with flehmen.

Although our observations in the mandrill may raise more questions than they answer, we note that our understanding of vertebrate olfactory perception is rapidly changing. For instance, contrary to long-held views about the separate and specialized functions of the main and accessory olfactory systems, there is increasing recognition that these two systems may interact to process sexual pheromones [Keller et al., 2009]. Likewise, a new class of chemosensory receptors, the trace amine-associated receptors (TAARs), have been shown to detect volatile molecules in mouse urine

[Liberles, 2009; Liberles & Buck, 2006], and TAARs recently have been discovered in the MOE of primates, including humans (see for review: [Liberles & Buck, 2006]). This new evidence suggests that Old World primates are equipped to decipher chemically encoded social and sexual information about conspecifics—a role previously ascribed to the defunct VNO. We cannot speculate on whether this new olfactory landscape will inform the mechanism of chemical communication in the mandrill, but the present findings identify the mandrill as an intriguing candidate for continued study.

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