2018

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Received: 8 March 2017 | Revised: 1 November 2017 | Accepted: 8 November 2017
PMID: 29473987
DOI: 10.1002/ajp.22730

RESEARCH ARTICLE

WILEY AMERICAN JOURNAL OF PRIMATOLOGY

Chemical composition of glandular secretions from a pair-living monogamous primate: Sex, age, and gland differences in captive and wild owl monkeys (*Aotus* spp.)

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Funding information
Zoological Society of San Diego; National Geographic Society; University of Pennsylvania Research Foundation; Wenner-Gren Foundation; National Science Foundation, Grant numbers: BCS-1232349, BCS-640 0621020, BCS-837921, BCS-904867, BCS-924352; International Primatological Society Research Grant; Amercian Society of Primatologists Small Research Grant; Leakey Foundation General Research Grant; Sigma-Xi Grant-in-Aid of Research; Nacey Maggioncalda Foundation James F. Nacey Fellowship

Broadening our knowledge of olfactory communication in strictly monogamous systems can inform our understanding of how chemosignals may facilitate social and reproductive behavior between the sexes. Compared to other social and mating systems, relatively little is known about olfactory communication in strictly monogamous non-human primates. Furthermore, platyrrhines are not well represented in chemical analyses of glandular secretions. We conducted semi-quantitative headspace gas chromatography with mass spectrometry to investigate the chemical components of glandular secretions from the subcaudal and pectoral glands of a strictly pair-living platyrrhine, the owl monkey (*Aotus* spp.). In this study, the first chemical analysis of a wild platyrrhine population, our goals were to (1) conduct a robust analysis of glandular secretions from both captive and wild owl monkey populations and (2) identify whether biologically relevant traits are present in glandular secretions. We also compared and contrasted the results between two *Aotus* species in different environmental contexts: wild *Aotus azarae* ($N = 33$) and captive *A. nancymaae* ($N = 104$). Our findings indicate that secretions from both populations encode sex, gland of origin, and possibly individual identity. These consistent patterns across species and contexts suggest that secretions may function as chemosignals. Our data also show that wild *A. azarae* individuals are chemically discriminated by age (adult or subadult). Among the captive *A. nanycmaae*, we found chemical differences associated with location, possibly caused by dietary differences. However, there was no noticeable effect of contraception on the chemical profiles of females, nor evidence that closely related individuals exhibit more similar chemical profiles in *A. nancymaae*. Overall, our data suggest that glandular secretions of both wild and captive *Aotus* convey specific information. Future studies should use behavioral bioassays to evaluate the ability of owl monkeys to detect signals, and consider whether odor may ultimately facilitate social and sexual relationships between male and female owl monkeys.

KEYWORDS
chemosignals, dynamic headspace analysis, monogamy, pair bonds, scent glands

1 | INTRODUCTION

Evidence of the critical role that chemosignals play in primate social behavior has been steadily increasing since the 1970s. In the past decade, research on non-human primate olfactory communication has flourished, dispelling the notion of the "microsmatic" primate (Heymann, 2006b; Laska & Salazar, 2015). Despite having smaller olfactory bulbs relative to brain size (Stephan, Baron, & Frahm, 1988) and a larger proportion of non-functioning olfactory receptor genes (Gilad, Bustamante, Lancet, & Pääbo, 2003; Gilad, Man, Pääbo, & Lancet, 2003; Rouquier, Blancher, & Giorgi, 2000; Young et al., 2002) compared to other mammals, these morphological differences in primates do not directly translate to differences in olfactory ability (Laska & Hudson, 1995; Smith & Bhatnagar, 2004). In fact, chemical evidence from non-human primate taxa suggest there are individual signatures of body odors secreted from scent glands, and that these odors encode information related to sex, age, rank, reproductive status, and genetic makeup (Drea, 2015). There is also substantial evidence that conspecifics can detect differences in these odors, and such odors may elicit behavioral or physiological changes in the odor recipient (Drea, 2015). More importantly, odor has been linked to variables (i.e., rank) important for mate choice in mandrills (Setchell, 2016), and are used in direct intra-sexual competition through stink-fights in ring-tailed lemurs (Jolly, 1966) and reproductive suppression in some callitrichines (Ziegler, 2013). As a first step to identify potential chemosignals in a strictly socially monogamous pair-living platyrrhine, we investigate the chemical components of glandular secretions in owl monkeys (*Aotus* spp.).

It seems likely that olfactory communication plays an integral role in intra-specific communication in owl monkeys that, like other platyrrhines, have scent glands (Hanson & Montagna, 1962; Hill, Appleyard, & Auber, 1959) and vomeronasal organs (Hunter, Fleming, & Dixson, 1984). Yet, among platyrrhines extensive research has been limited primarily to callitrichines (Heymann, 2006a). And while studies of callitrichines indicate that chemosignals affect both behavior and physiology of individuals by increasing sexual behavior based on fecundity cues in odor (Converse, Carlson, Ziegler, & Snowdon, 1995; Ziegler et al., 1993), suppressing ovulation of subordinate females (Barrett, Abbott, & George, 1990; Epple & Katz, 1984; Savage, Ziegler, & Snowdon, 1988), or modifying testosterone production in males (Ziegler, Peterson, Sosa, & Barnard, 2011), evidence of chemosignals are not yet available for most platyrrhine taxa. Moreover, only two published studies, in common marmosets ($N = 5$ individuals, Smith, 2006) and owl monkeys ($N = 13$ individuals, MacDonald, Fernandez-Duque, Evans, & Hagey, 2008) have investigated the chemical composition of glandular secretions in platyrrhines, and there have been no such studies of wild populations. This project is the first to chemically evaluate glandular secretions in platyrrhines with such a robust sample size, and the first to include a wild population.

The study also offers an opportunity to evaluate the glandular secretions of pair-living monogamous primates. To better understand the mechanisms and function of chemosignals in the context of mate choice throughout the primate clade, it is necessary to explore the function of putative chemosignals in different social and mating systems. To date, most studies have focused primarily on non-monogamous taxa, such as lemurs or mandrills, and cooperative breeders, such as callitrichines, all of which display different social and sexual relationships than those observed in owl monkeys. Owl monkeys are strictly socially monogamous, establishing multi-year relationships with no evidence of extra-pair reproduction (Huck, Fernandez-Duque, Babb, & Schurr, 2014). Given these differences in social and mating systems, it is reasonable to expect that chemosignals may function differently in owl monkeys than in non-monogamous taxa or species with more flexible mating relationships. When individuals form multi-year relationships, as in *Aotus*, an individual's reproductive success will be highly dependent on their breeding partner for several breeding seasons. In this case, we might expect that cues of individual quality are equally, or even more important, in pair-living taxa than in those for which the reproductive success of an animal is associated with mating with multiple partners. It is also possible that odor from glandular secretions are not primarily used to signal quality or traits used in mate choice, or to directly compete with conspecifics, but to facilitate the long-term bond between pair mates. Olfaction is an essential component of bonding in pair-living socially monogamous prairie voles (*Microtus ochrogaster*), where the removal of the vomeronasal organ or the olfactory bulb diminishes the development of partner preference between individuals (Curtis, Liu, & Wang, 2001; Williams, Slotnick, Kirkpatrick, & Carter, 1992). Identifying how chemosignals function in pair-living, socially monogamous taxa can help elucidate whether olfactory communication, and the associated physical traits, operate similarly across primate social and mating systems, or instead, whether they represent derived traits.

In this study, our goals were to (1) conduct a robust semi-quantitative analysis of glandular secretions from both captive and wild owl monkey (*Aotus* spp.) populations, including the first analysis of samples from a wild platyrrhine population and (2) identify whether biologically relevant traits are present in the glandular secretions of *Aotus*. We also used the two populations to compare and contrast results between two *Aotus* species in a captive (*Aotus nancymaae*) and wild (*A. azarae*) environmental context. Owl monkeys represent a good model species to investigate the potential role of olfactory communication in regulating male–female relationships and pair bonding. Anatomical and behavioral evidence strongly suggest olfactory communication is important for them. Anatomically, they possess both an olfactory bulb that is large relative to brain size and a vomeronasal organ (Hunter et al., 1984). They also have apocrine glands throughout the body (Hanson & Montagna, 1962), and a specialized subcaudal gland (Figure S1) with hypertrophic sebaceous and apocrine glands that exhibit thicker and more densely planted stiff, specialized hairs (Hanson & Montagna, 1962; Hill et al., 1959). Behaviorally, both captive and wild individuals regularly display patterns of scent-marking (rubbing scent glands on a substrate), partner-marking (rubbing scent glands on their pair mate), and inspecting (sniffing the anogenital/subcaudal region of their partner) (Wolovich & Evans, 2007). Experimental manipulations have shown that when male owl monkeys are deprived of olfactory cues,

aggressive interactions with unfamiliar males decrease (Hunter & Dixson, 1983). Finally, owl monkeys' glandular secretions are chemically rich, and it has been suggested by a study of a small number of individuals ($N = 13$), that they may contain information related to sex, age, and family group (MacDonald et al., 2008).

When considering our second goal of identifying biologically relevant information present in secretions, we hypothesized that olfactory cues in owl monkey body odor are used to communicate with potential mates. Specifically, we propose that these odors signal information that would be useful when choosing a partner. Under this hypothesis, we predicted that the odor of individuals would be statistically discriminated by sex and age category—as seen in a preliminary study of owl monkeys (MacDonald et al., 2008), lemurs (Greene & Drea, 2014; Morelli et al., 2013; Scordato, Dubay, & Drea, 2007), and mandrills (Setchell et al., 2010; Vaglio et al., 2016). Signals of relatedness may also be useful given the duration of owl monkey breeding relationships, the relatively infrequent opportunities for extra-pair mating, and the natal dispersal of males and females (Fernandez-Duque, 2009). Therefore, we predicted that close-kin dyads would have more similar chemical profiles than non-kin dyads, if inbreeding avoidance is mediated by olfactory cues, as is the case with socially monogamous beavers (Sun & Müller-Schwarze, 1998). Finally, if odors were individually identifiable, we would expect these signals to be somewhat stable over time and gland type, and predicted that there would be less intra-individual than inter-individual variation in chemical profile.

In addition to testing these four predictions, we also evaluated other variables not directly related to our hypothesis that may influence odor. First, given the differences in the frequency of scent-marking between the glands (Corley, Spence-Aizenberg, & Fernandez-Duque, in prep.; Spence-Aizenberg, Williams, & Fernandez-Duque, submitted.; Wolovich & Evans, 2007), the appearance of the glandular secretions from these glands (Spence-Aizenberg et al., unpublished data) and the chemical differences of gland type found in ring-tailed lemurs (Scordato & Drea, 2007), we evaluated whether secretions originating from the subcaudal and pectoral gland could be discriminated statistically. Additionally, we examined whether individuals could be statistically discriminated by location within the colony given some differences between colony rooms in the ambient environment or diet. We also tested for effects of contraception, which has been shown to alter the chemistry of secretions in lemurs (Crawford, Boulet, & Drea, 2011).

Finally, we have the ability to compare and contrast results across these taxa and differing environmental contexts by evaluating putative chemosignals in two different species and contexts,. The multi-year monitoring of wild (Owl Monkey Project, Argentina) and captive (Owl Monkey Breeding and Research Resource, DuMond Conservancy) owl monkey populations allow us to complement the intensive sampling and experimental approaches possible in captivity with ecological studies of wild individuals to better understand the adaptive value of putative chemosignals. A combined field-lab approach has already proved valuable in understanding food sharing (Wolovich, Feged, Evans, & Green, 2006; Wolovich & Perea-Rodriguez, 2007), mortality trajectories (Larson, Colchero, Jones, Williams, & Fernandez-Duque, 2016), and circadian biology (Fernandez-Duque, 2012; Fernandez-Duque, de la Iglesia, & Erkert, 2010). Similar patterns in these two populations would allow for more robust interpretations of the results than a study of only one species or environmental context.

2 | METHODS

2.1 | Study sites and subjects

We studied *Aotus nancymaae* ($N = 104$) housed at the Owl Monkey Breeding and Research Resource (OMBRR) located in the Keeling Center for Comparative Medicine and Research (MD Anderson Cancer Center, University of Texas, Bastrop). The OMBRR houses approximately 400 owl monkeys on a semi-reversed light cycle with periods of darkness extending approximately from 1500 to 0000 h. Animals are housed in one of two large colony rooms (North and South room), or a third smaller room. Animals are housed in pairs or family groups in enclosures approximately 1.8 m^3 in size, while a few individuals are housed alone. Water is always available to the animals, and they are fed LabDiet® Fiber-Plus® Monkey Diet 5049 (LabDiet; St. Louis, MO) with fruit or vegetable twice daily before 1500 h, which remains available throughout the dark cycle. While enclosures are directly adjacent to one another, groups are isolated visually from each other, and white noise (a waterfall) buffers the acoustic interactions within the rooms. Groups may be within olfactory range of their neighbors, but only in direct contact with their cagemates. Some adult females were administered monthly intra-muscular injections of a hormonal contraception ($N = 16$), medroxyprogesterone acetate (MPA). Because there were no marked differences in the gland secretion chemistry between non-contracepted and contracepted females (see below), samples from all females were included in the analyses.

We also studied a population of *Aotus azarae* ($N = 33$) ranging in gallery forests along the Pilagá and Guaycolec rivers in Formosa, Argentina (58° 11′W, 25° 58′S). This population has been monitored regularly since 1997 as part of the Owl Monkey Project. The low levels of sexual dimorphism in *Aotus* (Fernandez-Duque, 2011) make it necessary to mark individuals to reliably and regularly identify them. To do this, animals in this population are darted and anesthetized using ketamine hydrochloride projected from a CO_2-powered rifle and fitted with VHF radiocollars, or ball-chain collars with colored beads, to facilitate individual identification, following established methods (Fernandez-Duque & Rotundo, 2003; Juarez, Rotundo, Berg, & Fernandez-Duque, 2011).

This research on the captive *A. nancymaae* was approved by the MD Anderson Cancer Center Institutional Animal Care and Use Committee (ACUF# 05-13-04881). The Owl Monkey Project has had continued approval for all research on *A. azarae* presented here by the Formosa Province Council of Veterinarian Doctors, the Directorate of Wildlife, the Subsecretary of Ecology and Natural Resources and the Ministry of Production. At the national level, the procedures were approved by the National Wildlife Directorate in Argentina and by the IACUC committees of the Zoological Society of San Diego

(2000–2005) and of the University of Pennsylvania (2006–2013). This research adhered to the American Society of Primatologists principles for the ethical treatment of primates and the legal requirements of the United States.

2.2 | Data collection

One of us (ASA) collected 296 glandular secretions from 52 male and 52 female *A. nancymaae* during June–August 2013 (Table 1). Subjects ranged in age between 27 months and 25 years, and were defined as adults (>48 mos.) or subadults (24.1–48 mos.) (Huck, Rotundo, & Fernandez-Duque, 2011). The birthdates of two captive adults were unknown. We collected secretion samples from manually restrained animals by rubbing a sterile cotton swab over their subcaudal and/or pectoral scent gland back and forth five times following MacDonald et al. (2008). After collection, we sealed the swabs in a glass chromatography vial and stored them at −20°C (Drea et al., 2013; MacDonald et al., 2008). We collected a control swab (a swab exposed to the air) daily in each colony room where we sampled the animals. We shipped all samples on dry ice from the OMBRR to the University of Pennsylvania Reproductive Ecology Lab (Penn REL), where they were stored until analysis at the Monell Chemical Senses Center (Monell).

We also collected glandular secretions from 16 male and 17 female *A. azarae* wild individuals, with ages estimated between 16 months to 14 years, although seven adults were of unknown age. Their ages were defined as adults (>48 mos.), subadults (24.1–48 mos.), or juveniles (6.1-24 mos.) (Huck et al., 2011). Of the 72 samples collected from 33 individuals, we collected five (7%) of them between 2001 and 2007, and the remaining 67 (93%) between 2010 and 2013. We collected the scent gland samples while individuals were anesthetized for a physical exam conducted following their capture (Fernandez-Duque & Rotundo, 2003). Because captures require darting and anesthetization, we try to limit the number of individuals captured. Therefore, collection of glandular secretions are opportunistic and individuals may not contribute equally to the total sample. During physical exams, we rubbed sterile cotton swabs on the subcaudal and/or pectoral glands, stored them in separate glass vials, and transferred them to an off-site freezer within a few hours. We transported the samples at ambient temperature to the United States, then stored them at −20°C in the Penn REL until they were analyzed at Monell. We transferred the swabs to chromatography vials at Monell immediately prior to analysis.

2.3 | Data analyses

2.3.1 | Headspace analysis and identification

We conducted all odor analyses in B. Kimball's lab at Monell. We considered the *A. nancymaae* and *A. azarae* samples separately in both chromatographic and statistical analyses. To characterize the volatile components of collected secretions, we subjected the swabs to dynamic headspace analysis combined with gas chromatography-mass spectrometry (GC-MS). Headspace analyses were conducted with an HT3 dynamic headspace analyzer (Teledyne Tekmar, Mason, OH) using a Supelco Trap C desorption trap attached to a Thermo Trace GC-MS with a single quadrapole mass spectrometer and a 30 m 0.25 mm id Stabiliwax-DA fused-silica capillary column (RESTEK). Samples were maintained at 40°C, and swept with helium for 30 min at a 75 ml/min flow rate. Volatiles collected on the trap, which were desorbed at 180°C. The GC oven had an initial temperature of 40°C which was held for three min, then increased 7°C per minute to a final temperature of 230°C, which was held for 5.86 min. The MS was used in scan mode from 33 to 400 m/z. We used Xcalibur to convert the chromatographic data to NetCDF files, and Metalign (Lommen, 2009) for baseline correction, noise reduction, and peak alignment. We used MSClust (Tikunov, Laptenok, Hall, Bovy, & De Vos, 2012) to identify peaks, and to generate a chromatographic response based on chromatographic peak height. Empty vials and control samples were used to detect contaminants (Drea et al., 2013). We excluded from further analyses peaks with the largest peak heights in empty vials and control samples, as they were likely derived from the cotton swabs, chromatography vials, or the thermal desorption trap. Additionally, we

TABLE 1 Number of male and female individuals in the captive *A. nancymaae* and wild *A. azarae* populations from which subcaudal and pectoral gland secretion samples were collected

		Captive individuals		Wild individuals	
Sex	**Age**	**Subcaudal**	**Pectoral**	**Subcaudal**	**Pectoral**
Female	Adult	39	10	6	6
	Subadult	13	3	7	7
	Juvenile	–	–	1	2[a]
	Unknown	–	–	1	1
Male	Adult	33	5	8	11
	Subadult	19	4	4	3
	Juvenile	–	–	2[a]	2[a]
	Total	104	22	29	32

[a]One juvenile was also sampled as a subadult.

removed peaks detected in less than 10% of samples and duplicate peaks (representing the same compound). Peaks IDs are based on their scan number in the chromatogram (Table 2).

We calculated the relative abundance for the remaining peaks in ≥10% of samples (*N* = 110 peaks *A. nancymaae*, *N* = 70 peaks *A. azarae*) based on the sum of these peaks (referred to here as the total chromatogram area), allowing us to control for any variation in absolute abundance that might be due to the amount of secretion collected. We used these peak values to estimate chemical distances, with the values being square root transformed, centered, and scaled for all classification analyses to reduce the number of uni-variate outliers for all classification analyses. For peaks included in models, we confirmed identifies of eight peaks using authentic standards (Table 2, also see Supplementary Materials) and relied on tentative identifications provided by the NIST Standard Reference Database 1A (US Department of Commerce, Gaithersburg, MD) for all peaks we were not able to identify with standards.

Using principal component analysis, we identified outliers beyond the 95% confidence interval when plotting samples according to sample type using the first two components ("prcomp" function in R "stats" package, "ggord" in the package "ggplot2" in R). Identification and removal of outliers is critical when using linear discriminant analysis (LDA) because it is highly influenced by them. We had four samples in the captive data set (*N* = 2 females, 2 males), and four samples in the wild data set (*N* = 3 males, 1 female) whose values fell beyond the 95% confidence interval, and excluded these samples from statistical analyses. We conducted statistical analyses in R version 3.2.1 R (R Development Core Team, 2016).

2.3.2 | Classification of chemical data

To test whether glandular secretions encode information of age category, sex, gland type, and housing, we used these four variables as dependent variables in linear discriminant analysis (LDA), to assess how well the chemical content of gland secretions can accurately classify samples into the pre-existing categories (dependent variables) (Drea et al., 2013). Based on our predictions, we expected to statistically discriminate individuals in both populations based on sex and age. When testing the classification of sex and age categories (adult: >48 mos. or subadult: 24.1–48 mos.) (Huck et al., 2011), we used only subcaudal samples in the captive populations, but pooled the subcaudal and pectoral samples in the wild population because of the

TABLE 2 Peak ID, retention time, compound identification, and spectral match certainty of identification (between parentheses) for peaks used in LDA models for samples of captive *A. nancymaae* and wild *A. azarae*

Species	Peak	Retention time (min)	Model	Identified compound (%)
A. nancymaae	598	6.0	Location	2-Pentanone (90)
	667	6.4	Age category	Unknown
	1,053	**8.3**	**Sex**	**4-Heptanone**
	1,085	8.5	Gland type, location	Unknown
	1,297	**9.6**	**Gland type**	**2-Heptanone**
	1,448	**10.3**	**Location**	**2-Pentyl-furan**
	1,865	**12.5**	**Age category**	**4-Nonanone**
	2,453	15.4	Sex	Unknown
	2,507	15.7	Location	Unknown
	2,718	**16.8**	**Age category**	**Benzaldehyde**
	2,764	17.0	Gland type	4-Acetyl-1-methylcyclohexene
	3,473	20.6	Sex	Azulene[a] (36)
	3,887	22.7	Age category	trans-Shisool (30)
A. azarae	**1,392**	**10.1**	**Gland type**	**1-Butanol**
	1,674	11.5	Gland type	2,3,3-trimethyl-Cyclobutanone (48)
	2,379	15.1	Age category	Unknown
	2,713	16.7	Sex	Unknown
	2,977	**18.1**	**Age category**	**Linalool**
	3,867	22.6	Age category	1-(2-butoxyethyoxy)-ethanol (49)
	4,892	27.78	Gland type	5-Isoxazolecarboxylic acid (53)
	4,964	**28.15**	**Age category**	**4-Ethyl-phenol**

Compounds in bold were positively identified using standards (see Supplemental Materials).

[a]The likelihood that this peak is azulene is likely much higher, as the NIST Library identified this peak as azulene or naphthalene, and naphthalene was ruled out as the compound at this peak (see Supplemental Materials).

relatively small number of sampled individuals. We limited the analysis of gland type (subcaudal or pectoral) to adult and subadults, excluding the wild *A. azarae* juveniles because the number of subjects were so few. Location within the captive colony (North or South room) was used as a dependent variable in the LDA to evaluate signals of housing, and the samples were limited to the subcaudal secretions of individuals only housed in these two rooms. Additionally, to minimize the potential confounding factors of the predicted chemosignals of housing, age, and sex, we balanced, as much as possible, the number of individuals of each age and sex sampled in each room (North room: 30 adults,13 subadults, 22 males, 21 females; South room: 34 adults, 8 subadults 18 males, 24 females).

To conduct the LDAs, we first controlled for pseudo-replication of samples in the cases where multiple samples of the same gland had been collected from the same individual, to avoid increasing the risk of a Type 1 error (Setchell et al., 2010). After finding no ability to discriminate samples based on the month in which it was collected among the *A. nancymaae* (samples could not be accurately sorted in a LDA based on collection month, with a correctness rate of only 52% using five peaks), we computed averages of peak values across each individual's repeated samples. For the *A. azarae* samples, only five individuals contributed multiple samples from the same gland. In these cases, samples were averaged. Two subadult *A. azarae* were also sampled as juveniles. In these cases, their juvenile samples were not included in calculating average individual values, and were treated as independent juvenile samples. We used transformed peak values to perform stepwise forward variable selection to identify the peaks that separated the groups most for each dependent variable ("greedy.wilks" function in the klaR package in R; Weihs, Ligges, & Raabe, 2005). The peaks selected during the stepwise process were incrementally added as variables in linear discriminant analysis (using the "lda" function in the "MASS" package; Venables & Ripley, 2002). We assessed how well each model classified individuals into groups by assessing the correctness rate:

$$\text{Correctness rate} = ((\text{correct group 1 classifications})/(n \text{ group } 1) + (\text{correct group 2 classifications})/(n \text{ group } 2))/2$$

All of the correctness rates that we report represent the leave-one-out cross-validated classification rate for the models, and refer to the percentage of samples correctly classified. We considered the best models to be those that generated the highest correctness rate with the fewest variables.

2.3.3 | Chemical distances

To evaluate whether relatedness, individual identity, and contraception status are encoded in glandular secretions, we used chemical distances to estimate variation in chemical profiles within and between individuals. Chemical distances (CD) between samples were generated by calculating the Euclidean distance for each possible sample dyad. Smaller values suggest that the chemical profile of the samples within a dyad are more similar, whereas larger values suggest greater differences between samples. Next, we compared the chemical distances between "groups" using the chemical distances generated for all dyads within the following groups: (a) males and females to assess sex differences in intra-sexual variation; (b) close-kin (parent-offspring or full-sibling dyads) and non-kin (individuals not sharing any grandparents) to evaluate relatedness; (c) intra- and inter-individual to test individual identity over time (captive) and across gland type (wild); (d) subcaudal and pectoral (wild) to compare variation based on gland type; (e) North room and South room (captive) to estimate variation within colony rooms; (f) contracepted and non-contracepted females (captive) to evaluate contraception (Table 3 details each comparison, samples used, and dyads excluded from each analysis). Based on our predictions, we expected to find smaller CDs for close-kin than non-kin dyads, and for intra-individual than inter-individual dyads. We also expected to find smaller CDs among contracepted females than non-contracepted females given that they experience less hormonal fluctuation.

Because these data did not satisfy the criteria for assumptions of normality, we used the non-parametric Wilcoxon Rank Sum test to inferentially compare the chemical distances between groups, and we calculated the effect size "*r*," using the "rFromWilcox" function (Field, Miles, & Field, 2012). As with the classification analyses, we used average relative values of peaks for each individual to calculate CDs, except in the case of inter- and intra-individual comparisons, in which we used all samples.

3 | RESULTS

We identified 110 peaks endogenous to the subcaudal (*N* = 274) and pectoral samples (*N* = 22) collected from 104 captive *A. nancymaae* individuals and 70 peaks in the subcaudal (*N* = 37) and pectoral (*N* = 35) samples collected from 33 wild *A. azarae* individuals. For both the captive and wild data sets, the total area of the chromatogram, representing the total abundance of compounds detected, was greatest in the subcaudal glands, and lowest in the blank and control vials (Figures S2 and S3).

3.1 | Classification of glandular secretions

Male and female glandular secretions in both populations differed chemically. *A. nancymaae* individuals were accurately classified in the LDA model with 89% accuracy and *A. azarae* individuals were correctly classified by sex 69% of the time (Table 4 and Figure 1). Females were more accurately classified than males in both populations (Table 4).

Chemical differences in adult and subadult secretions were more apparent in the *A. azarae* than the *A. nancymaae*, with correctness rates of 76% and 60%, respectively (Table 4 and Figure 1).

Secretions from pectoral and subcaudal samples of owl monkeys differed markedly in their chemical composition. Samples were classified with 89% and 75% accuracy in the *A. nancymaae* and *A. azarae* populations respectively (Table 4 and Figure 1).

TABLE 3 Description of samples included and dyads excluded from all chemical distance analyses

Species	Dyad comparison	Sample type(s)	Excluded from analyses
A. nancymaae	M-M vs. F-F	SC-SC	M-F dyads; intra-individual dyads
	Close-kin vs. non-kin	SC-SC	Intra-individual dyads; individuals not associated with a family group
	Intra- vs. inter-individual	SC-SC	M-F dyads
	North vs. south room	SC-SC	Intra-individual dyads
	Non-[a] vs. contracepted Fs	SC-SC	Intra-individual dyads
	All dyads	SC-SC	None
A. azarae	M-M vs. F-F	SC-SC	M-F dyads; intra-individual dyads
	M-M vs. F-F	PE-PE	M-F dyads; intra-individual dyads
	Intra- vs. inter-individual	SC-PE	M-F dyads
	Subcaudal vs. pectoral	SC-SC, PE- PE	Intra-individual dyads
	All dyads	SC-SC, SC-PE, PE-PE	None

Results of the comparisons between chemical distances are in Table 5.

[a]Non-, non-contracepted females; SC-SC, subcaudal–subcaudal sample dyads; SC-PE, subcaudal-pectoral sample dyads; PE-PE, pectoral–pectoral sample dyads; M-M, male–male sample dyads; F-F, female–female sample dyads; Fs, females.

Location within the colony (North or South room) was also associated with differences in the chemical profile of *A. nancymaae* subcaudal secretions, with a correctness rate of 81% (Table 4). When this model was used to classify control samples according to the rooms in which they were sampled, control swabs ($N = 21$) were classified correctly only 61% of the time.

3.2 | Chemical distances (CDs)

We observed marked sex differences in CD when comparing same sex dyads. The median CD between male–male dyads was greater than that observed in female–female dyads for both *A. nancymaae* subcaudal, *A. azarae* subcaudal, and *A. azarae* pectoral secretions (Table 5). All these differences reached statistical significance, but the magnitude of difference was greater between the sexes in *A. azarae* than in *A. nancymaae*.

Close-kin dyads did not have more similar chemical profiles than non-kin dyads in *A. nancymaae* and the differences were not statistically significant (Table 5).

Chemical distances of samples from the same individual were smaller than CDs from different individuals in *A. nancymaae* and *A. azarae*. The median CD of intra-individual dyads was less than inter-individual dyads among the *A. nancymaae* subcaudal samples (Table 5). Among the *A. azarae*, the median CD between subcaudal and pectoral samples from the same individual were lower, although not statistically

TABLE 4 Peaks included in the best performing linear discriminant analysis model, correctness rate, and classification summary of glandular secretions from the subcaudal and pectoral samples obtained from captive *A. nancymaae* and wild *A. azarae*

Species	Category (sample type)	Peaks included[a]	Correctness rate	Correctly assigned (group type)	Incorrectly assigned (group type)
A. nancymaae	Sex (SC)	1,053, 2,453, 3,473	89%	51 (females) 42 (males)	1 (females) 10 (males)
	Age (SC)	1,865, 667, 3,887, 2,718	60%	64 (adults) 10 (subadults)	8 (adults) 22 (subadults)
	Gland type (SC, PE)	1,085, 1,297, 2,764	89%	101 (SC) 18 (PE)	3 (SC) 4 (PE)
	Location (SC)	1,085, 598, 1,448, 2,507	81%	37 (North room) 32 (South room)	6 (North room) 10 (South room)
A. azarae	Sex (SC & PE)	2,713	69%	23 (females) 19 (males)	8 (females) 11 (males)
	Age[b] (SC & PE)	4,964, 3,867, 2,379, 2,977	76%	28 (adult) 13 (subadult)	3 (adult) 8 (subadult)
	Gland type (SC & PE)	1,674, 4,892, 1,392	75%	21 (SC) 25 (PE)	8 (SC) 7 (PE)

SC, subcaudal; PE, pectoral.

[a]See Table 2 for tentative identity of each peak.

[b]Excluding wild juveniles.

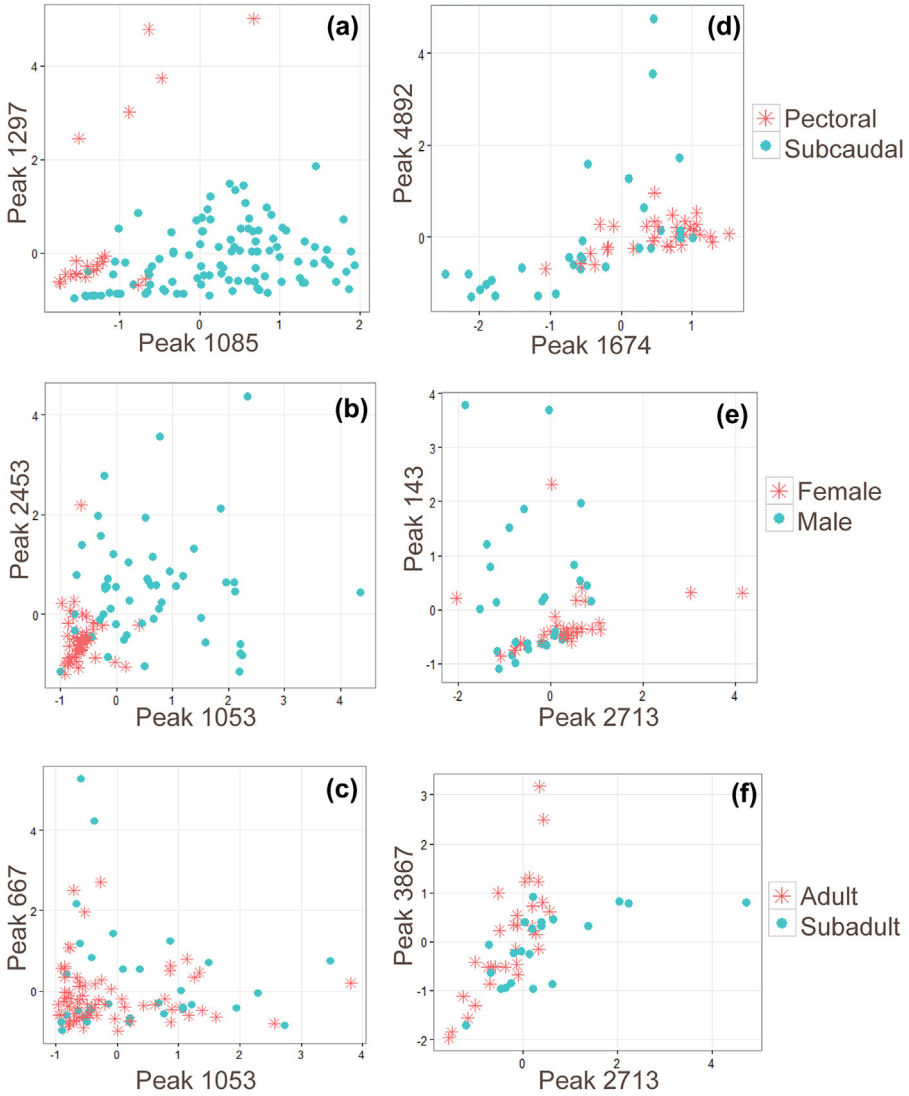

FIGURE 1 Individual averages of square-root transformed and scaled relative peak values for the first two peaks in the LDA model to discriminate captive *A. nancymaae* by (a) gland type, (b) sex, and (c) age category, and wild *A. azarae* by (d) gland type, (e) sex, and (f) age category

significantly different, than the median CD of subcaudal and pectoral samples from different individuals (Table 5).

We also observed differences in CD based on gland type in the *A. azarae* and housing location in the *A. nancymaae*; these differences reached statistical significance. On the other hand, there were no differences between the medians of females on or off contraception. Among the *A. azarae*, CDs between subcaudal secretions were much larger than CDs between pectoral secretions (Table 5). Captive *A. nancymaae* individuals housed in the North room had more similar chemical profiles than individuals in the South room (Table 5). There were no differences in the median CDs between contracepted and non-contracepted captive *A. nancymaae* females (Table 5).

4 | DISCUSSION

Our study suggests that owl monkey glandular secretions encode biologically relevant information. We found similar patterns in the glandular secretions of two owl monkey species, *A. azarae* and *A. nancymaae*, each in a different environment, wild and captivity. These patterns are positively related to sex, age, individual identity, gland type, and housing, suggesting that information is encoded in glandular secretions, which may act as chemosignals. The fact that these putative signals were reliably observed in two species, despite the differences in the data sets, speaks strongly of a biological relevance.

As predicted, there were consistent sex differences in the chemical composition of glandular secretions in both taxa, confirming the chemical dimorphism found in a preliminary study of a smaller population of captive *A. nancymaae* (MacDonald et al., 2008). While an olfactory sex signal in a primarily nocturnal taxon is not surprising in and of itself, it is particularly notable given that there have been virtually no reports in owl monkeys of conspicuous, marked, or seemingly biologically meaningful sex differences in size, body mass, growth development, dispersal patterns, fur coloration (Fernandez-Duque, 2011), and even close inspection of their external genitalia

TABLE 5 Medians, effect sizes, and statistical tests of differences in chemical distances of subcaudal secretion samples in captive *A. nancymaae* dyads and subcaudal and pectoral secretions samples in wild *A. azarae* dyads

Species	Dyad comparison	Median Euclidean distance (range)	*N* dyads	Effect size (*r*)	Wilcoxon rank sum (*W*)	*p*-value
A. nancymaae	M-M vs. F-F	M-M: 0.24 (0.08–0.71)	1,275	−0.131	745,050	<0.001
		F-F: 0.22 (0.09–0.45)	1,378			
	Close-kin vs. non-kin	Close-kin: 0.23 (0.11–0.64)	164	−0.020	211,770	0.31
		Non-kin: 0.23 (0.08–0.70)	2,466			
	Intra- vs. inter-individual	Intra-: 0.29 (0.13–0.67)	195	−0.025	1,657,400	<0.01
		Inter-: 0.31 (0.08–0.84)	15,262			
	North vs. south room	North: 0.19 (0.08–0.37)	903	−.436	192,700	<0.001
		South: 0.25 (0.12–0.71)	861			
	Non-[a] vs. contracepted Fs	Non-: 0.23 (0.09–0.45)	277	−0.014	16,337	0.79
		Contra-: 0.23 (0.13–0.37)	120			
	All dyads	0.32 (0.08–0.84)	5,356	n/a	n/a	n/a
A. azarae	M-M vs. F-F (SC)	M-M: 0.54 (0.15–0.97)	90	−0.434	2,345	<0.001
		F-F: 0.23 (0.08–0.89)	105			
	M-M vs. F-F (PE)	M-M: 0.25 (0.06–0.76)	119	−0.286	4,734	<0.001
		F-F: 0.16 (0.05–0.78)	119			
	Intra- vs. inter-individual	Intra-: 0.33 (0.09–0.89)	26	−0.016	5,887	0.726
		Inter-: 0.35 (0.06–1.02)	435			
	Subcaudal vs. pectoral	SC: 0.49 (0.07–1.00)	405	−0.394	54,293	<0.001
		PE: 0.21 (0.04–0.88)	494			
	All dyads	0.33 (0.04–1.02)	1,830	n/a	n/a	n/a

[a]Non-: non-contracepted females; M-M: male–male sample dyads; F-F: female–female sample dyads; Fs: Females; SC: subcaudal dyads; PE: pectoral dyads.

(Spence-Aizenberg et al., submitted). In addition to sex differences in the chemical composition of glandular secretions, we also estimated marked and consistent sex differences in the chemical distances. In both the captive and the wild populations, both the pectoral and subcaudal secretions of female–female dyads were more similar (i.e., had a smaller CD) than those of male–male dyads. This finding suggests that putative chemosignals among male owl monkeys varies more than among females. Given that dimorphism and variation of the dimorphic trait are two of the requirements to identify sexually selected traits (Snowdon, 2004), these results support the hypothesis that traits associated with the production of secretions in owl monkeys may be sexually selected traits, as have been proposed for other primate taxa (Drea, 2015; Heymann, 2003).

The chemical composition of the glandular secretions varied with age. While the model for age category performed well, with greater than 75% accuracy for the wild samples—comparable to what has been reported for male mandrills (Setchell et al., 2010; Vaglio et al., 2016)—it did not perform as well, with 60% accuracy, for the captive ones. Given the characteristics of our datasets, the performance of these models highlight the need to reflect on the criteria that our project uses to define age categories. In our analyses we relied on age categories of adult (>48 mos.) and subadult (24.1–48 mos.) that were established considering the age of immigration (approximately 4 years old) and age at first reproduction (never before 4 years old) within a wild population of *A. azarae* (Huck et al., 2011). However, this differs from our observations of captive subjects in a related study, in which an *A. nancymaae* breeding pair had an age of first reproduction as early as 38 months (male) and 45 months (female; Spence-Aizenberg et al., unpublished data). The age categories of adult and subadult used by our project are not defined in relation to reproductive development or maturity. Yet, evidence suggests that reproductive function is likely linked to the development and use of the subcaudal gland. For example, immature *Aotus* do not have well-developed subcaudal glands (Hill et al., 1959), but the administration of testosterone to a captive male less than 1 year old was correlated with an earlier development of this gland (Dixson, Gardner, & Bonney, 1980). In our study, the juvenile and subadult (<48 mos) *A. azarae* samples had a total abundance of chemical compounds in their chromatograms approximately 35% less than in adults, whereas the mean total abundance for the subadult *A. nancymaae* were comparable to adult *A. nancymaae* (7% less total abundance). The lower abundance suggests either a lower amount of secretion produced, and/or a less chemically rich secretion. If glandular development is correlated with rising levels of reproductive hormones, then age categories defined by life history traits in a wild population may not be biologically relevant in the context of olfactory communication and glandular development. Furthermore, recent research on wild *A. azarae* shows that subadult females exhibit reproductive hormones at levels similar to those of adults (Corley,

Valeggia, & Fernandez-Duque, 2017). This, combined with the reproductive success of subadults in captivity, suggests that the captive and wild individuals we categorize as subadults may span a range of reproductive functioning, and highlights a need to reevaluate the criteria used to define age categories.

Owl monkeys apparently have short-term individual signatures of odor. We conclude this based on the similarity of chemical profiles within individuals—over the course of 2–3 months in the captive population and across pectoral and subcaudal glands within an individual in the wild population—when compared to variation between individuals. Evidence for signals of individual identity in glandular secretions have been found in marmosets (Smith, 2006), ring-tailed lemurs (Scordato et al., 2007), and mandrills (Setchell et al., 2010). An ability to recognize individual identity encoded in odor would be useful in both territory defense and pair-bonding. Scent-marks from unfamiliar individuals would signal the presence of extra-group solitary individuals, potentially promoting territory defense. Additionally, the ability to recognize an individual's odor may facilitate the pair-bonding process. Odor plays a critical role in pair formation among socially monogamous prairie voles (*Microtus ochrogaster*); the removal of the vomeronasal organ, or the olfactory bulb, diminishes the development of partner preference (Curtis et al., 2001; Williams et al., 1992). In common marmosets, individuals can be conditioned to sexual arousal using an arbitrary odor (Snowdon, Tannenbaum, Schultz-Darken, Ziegler, & Ferris, 2011). It is possible, then, that owl monkeys become familiar with, and conditioned to, the individual odors of potential partners during the pair formation process, ultimately facilitating pair bonding.

The secretions produced by the pectoral and subcaudal gland were chemically distinct in both taxa. This is not surprising as there are marked differences in the frequency with which these glands are used in scent-marking, and that the secretions differ in color and amount, with the pectoral gland secreting a colorless secretion, while the subcaudal gland was typically secreting a dark, oily secretion in much greater amounts (Spence-Aizenberg et al., unpublished data). That individuals sniff the chest of group members but rarely scent-mark with the pectoral gland suggests that it may be used primarily for close-contact communication, likely serving a different function than the subcaudal gland. Our observations parallel those described for ring-tailed lemurs, where different glands are associated with differences in the chemical profiles and color of the glandular secretions (Scordato & Drea, 2007).

There was no evidence for a chemosignal of relatedness. Contrary to our predictions, there were no substantial differences in the overall chemical profile of close-kin and non-kin dyads. Our results also contradict a previous study reporting familial differences in owl monkey odor (MacDonald et al., 2008), although the small number of individuals used in this earlier study represented only three family groups who were also housed together. Therefore, the differences in that study may represent environmental, rather than familial, differences. While we found no evidence of chemosignals of kinship, it may be that some patterns of relatedness in secretions were obscured as we used pedigree, rather than genotype, to estimate relatedness. Pedigree was not found to correlate statistically with chemical distance in mandrills (Setchell et al., 2011), but relatedness based on genotype was found to correlate with chemical distances during the breeding season in ring-tailed lemurs (Boulet, Charpentier, & Drea, 2009; Boulet, Crawford, Charpentier, & Drea, 2010; Charpentier, Boulet, & Drea, 2008). Alternatively, it may be that relatedness may not be as important in mate choice as other genetic components. For instance, chemical distances in mandrill secretions were statistically significantly correlated with MHC dissimilarity (Setchell et al., 2011), and individual heterozygosity is correlated with the diversity of fatty acids in ring-tailed lemur labial secretions (Boulet et al., 2010). Moreover, although chemical analyses have identified volatile compounds associated with MHC type in mice, and mice can behaviorally differentiate between MHC types using urinary odor (Kwak et al., 2008), there is cross-study variation of the volatiles associated with MHC type in mice. It is likely then, that some aspects of odor perception cannot readily be evaluated by chemical measurements of volatile organic compounds even when the behavioral responses to odor variants are robust, as is the case with MHC type in mice (Kwak, Willse, Preti, Yamazaki, & Beauchamp, 2010). Ongoing research to assess the ability of owl monkeys to perceive relatedness through olfactory cues (Spence-Aizenberg, Kimball, Williams, & Fernandez-Duque, in prep.) will provide additional insights into the possible role of kinship recognition in regulating olfactory communication in owl monkeys.

There were mixed influences of housing and management on the chemical profile of captive individuals. Contraception had little to no effect on the odor of females, whereas location within the colony had a profound effect. Increased similarity in the chemical profiles of females receiving contraception would indicate that it altered the chemical profile so that there would be convergence among contracepted females, as has been reported for ring-tailed lemurs (Crawford et al., 2011). Surprisingly, the negligible differences in chemical profiles between non-contracepted and contracepted *A. nancymaae* females suggest contraception does not much alter the overall volatile chemical composition of subcaudal glandular secretions, despite the expected hormonal differences in females receiving contraception. Additionally, contraception does not impede the ability of females to form new pairs with males (L. Williams, personal communication), suggesting that the volatile metabolome was not drastically altered. However, within individual comparisons would improve the robusticity of these results.

The important chemical differences between samples from individuals housed in different colony rooms merit explanation. The most likely cause is environmental as there are no obvious sex or age differences in the animals sampled from these two rooms. Other environmental factors, including the standard diet and cleaning protocols, were the same in both rooms, and ambient environment is unlikely the cause as the control samples collected in each room could not be discriminated based on location. Therefore, the most evident environmental difference is dietary, as one room was receiving a diet supplemented with peanut butter while the other room did not. Given that the diet, and protein sources in particular, can influence body odor (Ferkin, Sorokin, Johnston, & Lee, 1997; Havlicek & Lenochova, 2006), the dietary peanut butter supplements are the most

plausible explanation for the chemical differences between animals in these two locations. Some of the compounds tentatively identified likely derived from diet. Specifically, 2-pentyl-furan—the identity of one of the compounds in the model for location—is not known to derive from mammalian metabolism and likely derives from diet according to the Pubchem online database (National Center for Biotechnology Information, CID = 19602).

When comparing results across species and contexts, we found that the models tended to less accurately classify wild *A. azarae* than captive *A. nancymaae*. While it is possible this is due to species differences, it seems more likely that differences in environment, sample handling, and data analysis contributed to increased variability in the *A. azarae* samples, reducing the ability to discriminate biologically meaningful variables. For instance, individuals in the wild have greater variation in diet both between groups (van der Heide, Fernandez-Duque, Iriart, & Juárez, 2012) and throughout the year (Fernandez-Duque, Rotundo, & Ramirez-Llorens, 2002). Additionally, samples collected in the field were not maintained continuously at freezing temperatures until arrival to the laboratory in the United States; changes in temperature are associated with a loss of volatiles in other taxa (Drea et al., 2013; Hayes, Morelli, & Wright, 2006). A potential loss of volatiles may be the reason for our finding that the samples from captive individuals were chemically richer than those from wild ones, with approximately 1.5 times the number of endogenous peaks. Finally, there were fewer wild individuals sampled than captive ones, which meant that we had to pool subcaudal and pectoral secretions, making it more difficult to identify other traits potentially causing variation in odor. Differences between the performances of models notwithstanding, the similarity in many of the results reinforces the notion that there are biologically meaningful patterns in the data.

In summary, it is hardly surprising that owl monkey odors encode information given the nocturnal habits of the taxon, the near absence of sexual dimorphism in physical features, and the frequency with which they engage in olfactory social behaviors. In both the captive *A. nancymaae* and wild *A. azarae* samples we found evidence for putative signals reported in other non-human primate taxa, including sex, age, individual identity, and gland type, but not for relatedness nor contraception status.

We have identified volatile compounds as putative signals in glandular secretions of owl monkeys, but this is only one component of the study of olfactory communication. Without confirming that these putative signals are perceived, we cannot identify them as chemosignals. Our ongoing implementation of behavioral bioassays and behavioral, hormonal, and olfactory monitoring of breeding pairs will complement the research presented here by addressing other facets of olfactory communication in *Aotus*. Beyond this, future work incorporating genetic measures of relatedness, non-volatile chemical cues in glandular secretions and urine, coupled with a better understanding of mate choice and the pair formation process, will surely contribute to a more comprehensive understanding of the role of olfactory communication in forming and maintaining male–female relationships, and how these processes may differ from non-monogamous taxa.

ACKNOWLEDGMENTS

This research was funded by the Leakey Foundation, International Primatological Society, American Society of Primatology, Sigma-Xi, and Nacey Maggioncalda Foundation grants to ASA, and grants from the National Science Foundation (BCS-1232349, BCS-640 0621020, BCS-837921, BCS-904867, BCS-924352), Wenner-Gren Foundation, National Geographic Society, the University of Pennsylvania Research Foundation, and the Zoological Society of San Diego to EFD. We would like to thank the Keeling Center for Comparative Medicine and Research (MD Anderson Cancer Center), Director Dr. C. Abee, and veterinarian Dr. A. Brady for allowing us access to the owl monkey colony. We would also like to thank G. Tustin, the owl monkey staff at the Keeling Center, E. Yau, and A. Fogel for assistance with sample collection at the Keeling Center, and to all of the students, volunteers, and assistants of the Owl Monkey Project who helped collecting the data in Formosa, Argentina, with special thanks to M. Rotundo, V. Dávalos, and C. Juárez for their work with the project and for sample collection in Argentina. This research complied with all animal care regulations of the MD Anderson Cancer Center, and the Owl Monkey Project has had continued approval for all research presented here by the Formosa Province Council of Veterinarian Doctors, the Directorate of Wildlife, the Subsecretary of Ecology and Natural Resources and the Ministry of Production. At the national level, the procedures were approved by the National Wildlife Directorate in Argentina and by the IACUC committees of the Zoological Society of San Diego (2000–2005), of the University of Pennsylvania (2006–2013), and of the MD Anderson Cancer Center (2013). Thanks to Mr F. Middleton and Ing. A. Casaretto of Bellamar Estancias S.A. and the Ministerio de la Producción y Ambiente of Formosa Province for the continued support of the Owl Monkey Project. The authors would also like to thank two anonymous reviewers for their helpful comments and suggestions.

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REFERENCES

Barrett, J., Abbott, D. H., & George, L. M. (1990). Extension of reproductive suppression by pheromonal cues in subordinate female marmoset monkeys, *Callithrix jacchus*. *Journal of Reproduction and Fertility*, *90*(2), 411–418.

Boulet, M., Charpentier, M. J. E., & Drea, C. M. (2009). Decoding an olfactory mechanism of kin recognition and inbreeding avoidance in a primate. *BMC Evolutionary Biology*, *9*, 281.

Boulet, M., Crawford, J. C., Charpentier, M. J. E., & Drea, C. M. (2010). Honest olfactory ornamentation in a female-dominant primate. *Journal of Evolutionary Biology*, *23*(7), 1558–1563.

Charpentier, M. J. E., Boulet, M., & Drea, C. M. (2008). Smelling right: The scent of male lemurs advertises genetic quality and relatedness. *Molecular Ecology*, *17*(14), 3225–3233.

Converse, L. J., Carlson, A. A., Ziegler, T. E., & Snowdon, C. T. (1995). Communication of ovulatory state to mates by female pygmy marmosets, *Cebuella pygmaea*. *Animal Behaviour*, *49*, 615–621.

Corley, M., Spence-Aizenberg, A., & Fernandez-Duque, E. (in prep). Olfactory communication in free-ranging owl monkeys: Natural history and spatial distribution of olfactory behaviors.

Corley, M., Valeggia, C., & Fernandez-Duque, E. (2017). Hormonal correlates of development and natal dispersal in wild female owl monkeys (*Aotus azarae*) of Argentina. *Hormones and Behavior*, *96*, 42.

Crawford, J. C., Boulet, M., & Drea, C. M. (2011). Smelling wrong: Hormonal contraception in lemurs alters critical female odour cues. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 122–130.

Curtis, J. T., Liu, Y., & Wang, Z. (2001). Lesions of the vomeronasal organ disrupt mating-induced pair bonding in female prairie voles (*Microtus ochrogaster*). *Brain Research*, *901*(1–2), 167–174.

Dixson, A. F., Gardner, J. S., & Bonney, R. C. (1980). Puberty in the male owl monkey (*Aotus trivirgatus griseimembra*): A study of physical and hormonal development. *International Journal of Primatology*, *1*, 129–139.

Drea, C. M. (2015). D'scent of man: A comparative survey of primate chemosignaling in relation to sex. *Hormones and Behavior*, *68*, 117–133.

Drea, C. M., Boulet, M., Delbarco-Trillo, J., Greene, L. K., Sacha, C. R., Goodwin, T. E., & Dubay, G. R. (2013). The "secret" in secretions: Methodological considerations in deciphering primate olfactory communication. *American Journal of Primatology*, *22*, 1–22.

Epple, G., & Katz, Y. (1984). Social influences on estrogen excretion and ovarian cyclicity in saddle back tamarins (*Saguinus fuscicollis*). *American Journal of Primatology*, *6*(3), 215–227.

Ferkin, M. H., Sorokin, E. S., Johnston, R. E., & Lee, C. J. (1997). Attractiveness of scents varies with protein content of the diet in meadow voles. *Animal Behaviour*, *53*, 133–141.

Fernandez-Duque, E. (2009). Natal dispersal in monogamous owl monkeys (*Aotus azarai*) of the Argentinean Chaco. *Behaviour*, *146*(4), 583–606.

Fernandez-Duque, E. (2011). Rensch's rule, Bergmann's effect and adult sexual dimorphism in wild monogamous owl monkeys (*Aotus azarai*) of Argentina. *American Journal of Physical Anthropology*, *146*, 38–48.

Fernandez-Duque, E. (2012). Owl monkeys *Aotus* spp. in the wild and in captivity. *International Zoo Yearbook*, *46*, 80–94.

Fernandez-Duque, E., de la Iglesia, H., & Erkert, H. G. (2010). Moonstruck primates: Owl monkeys (*Aotus*) need moonlight for nocturnal activity in their natural environment. *PLoS ONE*, *5*(9), 1–6.

Fernandez-Duque, E., & Rotundo, M. (2003). Field methods for capturing and marking Azarai night monkeys. *International Journal of Primatology*, *24*, 1113–1121.

Fernandez-Duque, E., Rotundo, M., & Ramirez-Llorens, P. (2002). Environmental determinants of birth seasonality in night monkeys (*Aotus azarai*) of the Argentinean Chaco. *International Journal of Primatology*, *23*(3), 639–656.

Field, A., Miles, J., & Field, Z. (2012). *Discovering statistics using R*. London: Sage Publications.

Gilad, Y., Bustamante, C. D., Lancet, D., & Pääbo, S. (2003). Natural selection on the olfactory receptor gene family in humans and chimpanzees. *American Journal of Human Genetics*, *73*, 489–501.

Gilad, Y., Man, O., Pääbo, S., & Lancet, D. (2003). Human specific loss of olfactory receptor genes. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 3324–3327.

Greene, L. K., & Drea, C. M. (2014). Love is in the air: Sociality and pair bondedness influence sifaka reproductive signalling. *Animal Behaviour*, *88*, 147–156.

Hanson, G., & Montagna, W. (1962). The skin of primates. XII The skin of the owl monkey (*Aotus trivirgatus*). *American Journal of Physical Anthropology*, *20*, 421–429.

Havlicek, J., & Lenochova, P. (2006). The effect of meat consumption on body odor attractiveness. *Chemical Senses*, *31*(8), 747–752.

Hayes, R. A., Morelli, T. L., & Wright, P. C. (2006). Volatile components of lemur scent secretions vary throughout the year. *American Journal of Primatology*, *68*, 1202–1207.

Heymann, E. W. (2003). Scent marking, paternal care, and sexual selection in callitrichines. In C. B. Jones (Ed.), *Sexual selection and reproductive competition in primates: New perspectives and directions* (pp. 305–325). Norman, OK: American Society of Primatologists.

Heymann, E. W. (2006a). Scent marking strategies of New World primates. *American Journal of Primatology*, *68*, 650–661.

Heymann, E. W. (2006b). The neglected sense-olfaction in primate behavior, ecology, and evolution. *American Journal of Primatology*, *68*, 519–524.

Hill, W. C. O., Appleyard, H. M., & Auber, L. (1959). The specialised area of skin glands in *Aotes Humboldt* (*Simiae platyrrhini*). *Transactions of the Royal Society of Edinburgh*, *63*, 535–551.

Huck, M., Fernandez-Duque, E., Babb, P., & Schurr, T. (2014). Correlates of genetic monogamy in socially monogamous mammals: Insights from Azara's owl monkeys. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140195.

Huck, M., Rotundo, M., & Fernandez-Duque, E. (2011). Growth and development in wild owl monkeys (*Aotus azarai*) of Argentina. *International Journal of Primatology*, *32*(5), 1133–1152.

Hunter, A., & Dixson, A. (1983). Anosmia and aggression in male owl monkeys (*Aotus trivirgatus*). *Physiology and Behavior*, *30*, 875–879.

Hunter, A., Fleming, D., & Dixson, A. F. (1984). The structure of the vomeronasal organ and nasopalatine ducts in *Aotus trivirgatus* and some other primate species. *Journal of Anatomy*, *138*, 217–225.

Jolly, A. (1966). *Lemur behavior*. Chicago: University of Chicago Press.

Juarez, C. P., Rotundo, M. A., Berg, W., & Fernandez-Duque, E. (2011). Costs and benefits of radio-collaring on the behavior, demography, and conservation of owl monkeys (*Aotus azarai*) in Formosa, Argentina. *International Journal of Primatology*, *32*(1), 69–82.

Kwak, J., Willse, A., Matsumura, K., Opiekun, M. C., Yi, W., Preti, G., … Beauchamp, G. K. (2008). Genetically-based olfactory signatures persist despite dietary variation. *PLoS ONE*, *3*(10), e3591.

Kwak, J., Willse, A., Preti, G., Yamazaki, K., & Beauchamp, G. K. (2010). In search of the chemical basis for MHC odourtypes. *Proceedings of the Royal Society B: Biological Sciences*, *2277*, 2417–2425.

Larson, S. M., Colchero, F., Jones, O. R., Williams, L., & Fernandez-Duque, E. (2016). Age and sex-specific mortality of wild and captive populations of a monogamous pair-bonded primate (*Aotus azarae*). *American Journal of Primatology*, *78*, 315–325.

Laska, M., & Hudson, R. (1995). Ability of female squirrel monkeys (*Saimiri sciureus*) to discriminate between conspecific urine odours. *Ethology*, *99*(1-2), 39–52.

Laska, M., & Salazar, L. T. H. (2015). Olfaction in nonhuman primates. In R. L. Doty (Ed.), *Handbook of olfaction and gustation* (pp. 605–621). Hoboken, NJ: John Wiley & Sons.

Lommen, A. (2009). MetAlign: Interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data preprocessing. *Analytical Chemistry*, *81*, 3079–3086.

MacDonald, E. A., Fernandez-Duque, E., Evans, S., & Hagey, L. R. (2008). Sex, age, and family differences in the chemical composition of owl monkey (*Aotus nancymaae*) subcaudal scent secretions. *American Journal of Primatology*, *18*, 12–18.

Morelli, T. L., Hayes, R. A., Nahrung, H. F., Goodwin, T. E., Harelimana, I. H., MacDonald, L. J., & Wright, P. C. (2013). Relatedness communicated in lemur scent. *Naturwissenschaften*, *100*(8), 769–777.

National Center for Biotechnology Information. (n.d.). PubChem Compound Database; CID=19602. Retrieved March 27, 2017, from https://pubchem.ncbi.nlm.nih.gov/compound/19602 Create date March 27, 2005.

R Development Core Team. (2016). R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

Rouquier, S., Blancher, A., & Giorgi, D. (2000). The olfactory receptor gene repertoire in primates and mouse: Evidence for reduction of the

functional fraction in primates. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 2870–2874.

Savage, A., Ziegler, T. E., & Snowdon, C. T. (1988). Sociosexual development, pair bond formation, and mechanisms of fertility suppression in female cotton-top tamarins (*Saguinus oedipus oedipus*). *American Journal of Primatology*, *14*, 345–359.

Scordato, E. S., & Drea, C. M. (2007). Scents and sensibility: Information content of olfactory signals in the ringtailed lemur, *Lemur catta*. *Animal Behaviour*, *73*, 301–314.

Scordato, E. S., Dubay, G., & Drea, C. M. (2007). Chemical composition of scent marks in the ringtailed lemur (*Lemur catta*): glandular differences, seasonal variation, and individual signatures. *Chemical Senses*, *32*(5), 493–504.

Setchell, J. M. (2016). Sexual selection and the differences between the sexes in mandrills (*Mandrillus sphinx*). *Yearbook of Physical Anthropology*, *159*, S105–S129.

Setchell, J. M., Vaglio, S., Abbott, K. M., Moggi-Cecchi, J., Boscaro, F., Pieraccini, G., & Knapp, L. A. (2011). Odour signals major histocompatibility complex genotype in an Old World monkey. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1703), 274–280.

Setchell, J. M., Vaglio, S., Moggi-Cecchi, J., Boscaro, F., Calamai, L., & Knapp, L. A. (2010). Chemical composition of scent-gland secretions in an Old World monkey (*Mandrillus sphinx*): Influence of sex, male status, and individual identity. *Chemical Senses*, *35*, 205–220.

Smith, T. (2006). Individual olfactory signatures in common marmosets (*Callithrix jacchus*). *American Journal of Primatology*, *68*, 585–604.

Smith, T. D., & Bhatnagar, K. P. (2004). Microsmatic primates: Reconsidering how and when size matters. *Anatomical Record. Part B, New Anatomist*, *279*(1), 24–31.

Snowdon, C. T. (2004). Sexual selection and communication. In P. Kappeler & C. P. van Schaik (Eds.), *Sexual selection in primates: New and comparative perspectives* (pp. 57–70). Cambridge, UK: Cambridge University Press.

Snowdon, C. T., Tannenbaum, P. L., Schultz-Darken, N. J., Ziegler, T. E., & Ferris, C. F. (2011). Conditioned sexual arousal in a nonhuman primate. *Hormones and Behavior*, *59*(5), 696–701.

Spence-Aizenberg, A., Kimball, B. A., Williams, L., & Fernandez-Duque, E. (in prep). Odor discrimination of glandular secretions in *Aotus nancymaae*.

Spence-Aizenberg, A., Williams, L., & Fernandez-Duque, E. (submitted). Are olfactory traits in a pair-bonded primate under sexual selection? An evaluation of sexual dimorphism in *Aotus nancymaae*.

Stephan, H., Baron, G., & Frahm, H. D. (1988). Comparative size of brains and brain components. In H. Steklis & J. Erwin (Eds.), *Comparative primate biology* (Vol. *4*, pp. 1–38). New York: Alan R. Liss.

Sun, L., & Müller-Schwarze, D. (1998). Anal gland secretion codes for relatedness in the beaver, *Castor canadensis*. *Ethology*, *104*, 917–927.

Tikunov, Y., Laptenok, S., Hall, R., Bovy, A., & De Vos, R. (2012). MSClust: A tool for unsupervised mass spectra extraction of chromatography-mass spectrometry ion-wise aligned data. *Metabolomics*, *7*, 714–718.

Vaglio, S., Minicozzi, P., Romoli, R., Boscaro, F., Pieraccini, G., Moneti, G., & Moggi-cecchi, J. (2016). Sternal gland scent-marking signals sex, age, rank, and group identity in captive mandrills. *Chemical Senses*, *41*(2), 177–186.

van der Heide, G., Fernandez-Duque, E., Iriart, D., & Juárez, C. P. (2012). Do forest composition and fruit availability predict demographic differences among groups of territorial owl monkeys (*Aotus azarai*)? *International Journal of Primatology*, *33*(1), 184–207.

Venables, W., & Ripley, B. (2002). *Modern applied statistics with S (Fourth)*. New York: Springer.

Weihs, C., Ligges, U., & Raabe, N. (2005). klaR analyzing German business cycles. In D. Baier, R. Decker, & L. Schmidt-Thieme (Eds.), *Data analysis and decision support* (pp. 335–343). Berlin: Springer-Verlag.

Williams, J. R., Slotnick, B. M., Kirkpatrick, B. W., & Carter, C. S. (1992). Olfactory bulb removal affects partner preference development and estrus induction in female prairie voles. *Physiology and Behavior*, *52*(4), 635–639.

Wolovich, C. K., & Evans, S. (2007). Sociosexual behavior and chemical communication of *Aotus nancymaae*. *International Journal of Primatology*, *28*, 1299–1313.

Wolovich, C. K., Feged, A., Evans, S., & Green, S. M. (2006). Social patterns of food sharing in monogamous owl monkeys. *American Journal of Primatology*, *674*, 663–674.

Wolovich, C. K., & Perea-Rodriguez, J. P. (2007). Food transfers to young and mates in wild owl monkeys (*Aotus azarai*). *American Journal of Primatology*, *16*, 1–16.

Young, J. M., Friedman, C., Williams, E. M., Ross, J. A., Tonnes-Priddy, L., & Trask, B. J. (2002). Different evolutionary processes shaped the mouse and human olfactory receptor gene families. *Human Molecular Genetics*, *11*(5), 535–546.

Ziegler, T. E. (2013). Social effects via olfactory sensory stimuli on reproductive function and dysfunction in cooperative breeding marmosets and tamarins. *American Journal of Primatology*, *75*, 202–211.

Ziegler, T. E., Epple, G., Snowdon, C. T., Porter, T. A., Belcher, A. M., & Küuderling, I. (1993). Detection of the chemical signals of ovulation in the cotton-top tamarin, *Saguinus oedipus*. *Animal Behaviour*, *45*, 313–322.

Ziegler, T. E., Peterson, L. J., Sosa, M. E., & Barnard, A. M. (2011). Differential endocrine responses to infant odors in common marmoset (*Callithrix jacchus*) fathers. *Hormones and Behavior*, *59*(2), 265–270.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Spence-Aizenberg A, Kimball BA, Williams LE, Fernandez-Duque E. Chemical composition of glandular secretions from a pair-living monogamous primate: Sex, age, and gland differences in captive and wild owl monkeys (*Aotus* spp.). *Am J Primatol*. 2018;80:e22730. https://doi.org/10.1002/ajp.22730