

FAST TRACK

Smelling right: the scent of male lemurs advertises genetic quality and relatedness

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Abstract

Sexual selection theory predicts that competitors or potential mates signal their quality or relatedness to conspecifics. Researchers have focused on visual or auditory modes of signal transmission; however, the importance of olfactory indicators is gaining recognition. Using a primate model and a new integrative analytical approach, we provide the first evidence relating male olfactory cues to individual genome-wide heterozygosity and to the genetic distance between individuals. The relationships between male semiochemical profiles and genetic characteristics are apparent only during the highly competitive and stressful breeding season. As heterozygosity accurately predicts health and survivorship in this population, we identify scrotal olfactory cues as honest indicators of male quality, with relevance possibly to both sexes. Beyond showing that semiochemicals could underlie kin recognition and nepotism, we provide a putative olfactory mechanism to guide male–male competition and female mate choice.

Keywords: genetic compatibility, heterozygosity, olfactory communication, semiochemical, sexual selection, strepsirrhine primate

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Introduction

Sexual selection research has provided growing empirical evidence that the competitive sex (typically the male) advertises his quality to potential opponents to minimize intrasexual contest and the choosy sex (typically the female) relies on these honest indicators to select an appropriate mate (Andersson 1994). The accurate assessment of genetic characteristics also may be critical for directing nepotism towards relatives (Sherman 1980) or for ensuring successful conception and viability of the offspring (Bateson 1978; Tregenza & Wedell 2002; Mays & Hill 2004; Hoffman *et al.* 2007). Two main hypotheses may explain how female choice increases offspring fitness. According to the 'good genes' hypothesis, specific cues honestly signal a male's genetic quality. According to the 'compatibility' hypothesis, genetic dissimilarity between mates increases the genetic

diversity and, hence, fitness of offspring (Mays & Hill 2004). Herein lies a paradox, however, as for most females, the male possessing good genes may not be the most genetically compatible. Resolution of this paradox therefore requires that both sources of information, quality and compatibility, be available. Using as a model the ring-tailed lemur (*Lemur catta*), a strepsirrhine primate endemic to Madagascar, we examine the genetic correlates of male signals.

Researchers have focused on visual or acoustic modes of signal transmission, such as weaponry, ornamentation, and song (Andersson 1994); however, the importance of olfactory indicators is gaining appreciation (Müller-Schwarze 2006). Moreover, there is increasing evidence that pheromonal traits are heritable (reviewed in Johansson & Jones 2007). Deciphering the chemical components contained in olfactory cues has provided important insights into the functional significance of mammalian scent marking (Albone 1984; Müller-Schwarze 2006). Through advances in semiochemistry, volatile and nonvolatile components of olfactory signals have been shown to vary, for example, by species (Belcher *et al.* 1986), sex or reproductive state (Belcher *et al.* 1986; Buesching *et al.* 2002; Zhang *et al.* 2005; Soini *et al.* 2007),

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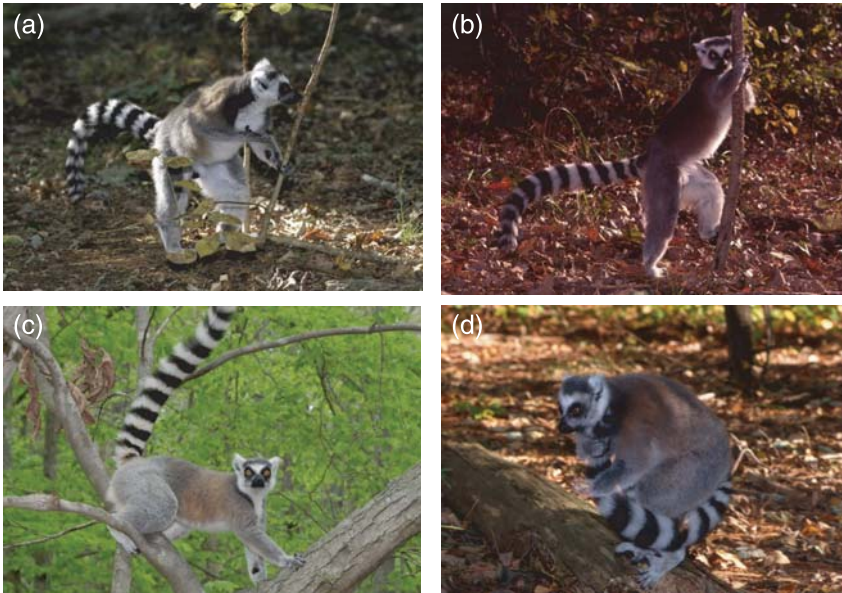


Fig. 1 Male ring-tailed lemur scent-marking behaviour: (a) shoulder rubbing, presumably to mix the brachial and antebrachial secretions before wrist marking; (b) wrist marking, which makes an audible clicking sound as the antebrachial spur scars the sapling; (c) scrotal or genital marking, for which the male assumes a hand-stand posture, and; (d) tail anointing, whereby a male impregnates his tail fur with secretions before wafting it at an opponent during a 'stink fight.' Photos courtesy of David Haring, Duke Lemur Center.

dominance status (Miller *et al.* 1998; Hayes *et al.* 2003), and even individual identity (Hurst *et al.* 2001; Zhang *et al.* 2005), bolstering suggestions that scent marking serves to transmit information about group membership, sexual receptivity, competitive ability, and resource ownership. Olfactory cues also have been implicated in the advertisement of kinship (Mateo 2003) and genetic compatibility between potential mates (Penn 2002), but the semiochemical link to genetic variation remains obscure.

In relation to kinship, semiochemical analyses conducted on family or clan members, using pedigree information or mitochondrial DNA markers, respectively, have revealed convergent semiochemical profiles among related individuals compared to unrelated individuals (Sun & Müller-Schwarze 1998b) and similar profiles along maternal lines (Safi & Kerth 2003), but no correlation with genetic distance, *per se*. In relation to genetic compatibility, studies of olfactory-guided discrimination in humans and other species typically rely on female responses to male odourants, as a proxy for mate choice, and/or address variation at genes of the major histocompatibility complex (MHC), specifically (Brown & Eklund 1994; Wedekind *et al.* 1995; Penn 2002; Roberts & Gosling 2003; Milinski *et al.* 2005; Parrott *et al.* 2007). Whereas behavioural bioassays reveal important information about female olfactory discrimination, their validity for reflecting mate choice remains uncertain; typically, such studies also fall short of deciphering the semiochemical mechanism underlying behavioural differences. Nevertheless, in relation to genetic diversity, Willse and colleagues conducted a revealing study on two inbred mouse lines and their heterozygous cross, showing that both MHC and 'background' genes influenced the animals' semiochemical profiles (Willse *et al.* 2006). If odourants communicate genetic characteristics, as suggested by these studies, we reasoned that scent signals

should systematically reflect more genome-wide variation, including heterozygosity and genetic distance. Here, we integrate current analytical approaches from chemistry, ecology, and genetics, in a novel way, to link a male's olfactory signals to these genetic characteristics.

The ring-tailed lemur represents an ideal model system for the study of olfactory communication. It lives in multimale-multifemale societies, characterized by female dominance and strict seasonal breeding (Jolly 1966), and possesses the most elaborate, multimodal scent-marking repertoire of any primate (Drea & Scordato 2007). Males have three species-specific glands (the scrotal, brachial, and antebrachial glands): each is used in different manners (Fig. 1), each produces distinct secretions that generate different responses, both under semi-free-ranging conditions and during behavioural bioassays, and each likely serves unique functions (Drea & Scordato 2007; Scordato & Drea 2007). Previously, using gas chromatography and mass spectrometry (GC-MS), we and others have shown that the volatile profile of each glandular secretion is unique (Hayes *et al.* 2005; Scordato *et al.* 2007), changes seasonally (Scordato *et al.* 2007), and varies by individual (Palagi & Dapporto 2006; Scordato *et al.* 2007). Although lemurs investigate all types of scent marks (Scordato & Drea 2007), we selected for the present analyses the scrotal secretions, as they are the most semiochemically complex of the three male secretions, less volatile than antebrachial secretions, more individually variable than brachial secretions, and most similar to labial secretions (Scordato *et al.* 2007), which ultimately will permit intersexual comparisons. Here, we couple GC-MS data with genetic analyses to test whether the seasonally variable volatile messages contained in the scrotal secretions of male ring-tailed lemurs carry information about the males' genetic constitution.

Methods

Subjects

The subjects were 19 adult, reproductively intact, male *Lemur catta*, aged 1.5–24 years, housed at the Duke Lemur Centre in Durham, North Carolina. In captivity, animals at our youngest ages are sexually mature (Drea 2007). Most subjects typically live in semi-free-ranging groups that occupy forested enclosures (ranging in size from 1.5 to 10.9 ha), each with access to indoor, heated rooms or thermostatically controlled nest boxes; however, animals may occupy smaller enclosures (278–347 ft²) with fewer companions or even alone. Details of the housing conditions have been provided elsewhere (Scordato & Drea 2007). In the Northern Hemisphere, where seasons are shifted by 6 months from those in Madagascar, the 'breeding season' spans from early November to early February (Drea 2007). Here, we considered all other times of the year as the 'nonbreeding season'.

The diet of our subjects is relatively stable throughout the year. Although the lemurs may forage on the native flora while free ranging, they primarily eat Purina Monkey Chow (Monkey PMI Feeds) and assorted fruits and vegetables. Using procedures described below, we have found that the GC–MS profiles of monkey chow and scrotal secretions are quite distinct, as most compounds secreted by the scrotal gland are not present in the food (Sacha *et al.* 2008). The animals were maintained in accordance with US Department of Agriculture regulations and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All research protocols were approved by the Institutional Animal Care and Use Committee of Duke University (protocol #A245-03-07).

Odourant sample collection, extraction, and GC–MS analyses

We collected samples of glandular secretion throughout the year, as before (Scordato & Drea 2007), by gently restraining subjects and rubbing pre-cleaned cotton swabs, held with clean forceps, repeatedly against the glandular area of the scrotum (at the base of the penis) and up into the two 'pockets' of this gland. We stored samples at –80 °C until extraction using the following modifications to our solvent-based protocol (Safi & Kerth 2003; Scordato *et al.* 2007): we concentrated the solvent extracts to 50–75 µL and added 5 µL of an internal standard (hexachlorobenzene, 1 mg/mL) to verify consistency of retention times between runs.

We analysed these extracts on a Shimadzu GCMS-QP2010 instrument (Shimadzu Scientific Instruments) equipped with a Shimadzu AOC-20 series autosampler. To improve the discrimination of compounds, we modified our prior parameters (Scordato *et al.* 2007) as follows: we increased

total run time to 43 min by ramping the temperature at a slower rate between 180 °C and 320 °C (5 °C/min). We detected semiochemicals using the automatic peak detector (SOLUTION WORKSTATION software, Shimadzu Scientific Instruments) and verified peaks individually by consulting the National Institute of Standards and Technology library.

We standardized the retention times (*rt*) of peaks with those of the internal standard (*rt* = 12.6 min) and squalene (*rt* = 28.5 min), a major component of scrotal secretions (Scordato *et al.* 2007). We then aligned peaks using the standardized *rt* and molecular weights of compounds. Whenever possible, we identified compounds based on mass spectra, *rt*, and prior tentative identification (Scordato *et al.* 2007), but contrary to prior analyses, we did not combine the relative areas of structural isomers because our modified GC–MS protocol allowed the separation of these compounds. For statistical analyses, we retained peaks (*n* = 203) that comprised at least 0.05% of the overall area of the chromatogram and had consistent standardized *rt* (Scordato *et al.* 2007). These compounds included 77 fatty acid esters, 25 fatty acids, 8 mixtures of co-eluting compounds, 3 cholesterol derivatives, 2 alkanes, 1,6,10-dodecatriene, 7,11-dimethyl-3-methylene (E), farnesol, squalene, and 85 unidentified compounds. Figure 2 provides a visual example of GC–MS chromatograms.

Chemical variables

To quantify and represent the semiochemical complexity of male scrotal secretions, we adopted a novel analytical approach for GC–MS data, generating the following major diversity indices typically used by community ecologists: richness, Shannon index, and Simpson index (Legendre & Legendre 1998; McCune *et al.* 2002). Here, richness refers to the total number of compounds per chromatogram and weights rare and common compounds equally. In contrast, both the Shannon and Simpson indices reflect the relative abundance of detected compounds, but are slightly different: whereas the Shannon index is most strongly influenced by the relatively common compounds that are of intermediate abundance, the Simpson index is more sensitive to compounds that show the greatest relative abundance, and hence is the index least affected by rare compounds (McCune *et al.* 2002). We used these measures first to better characterize the seasonal variation in semiochemical profiles and next as a metric against which to correlate genetic diversity (see below). We also used the relative abundances of the 203 chemical compounds to calculate relative Euclidean distances for each dyad of males (McCune *et al.* 2002). We used this measure in correlative analyses with genetic distance (see below). We calculated the three indices of chemical diversity and the chemical distances using the software PC-ORD 5.0 (McCune *et al.* 2002).

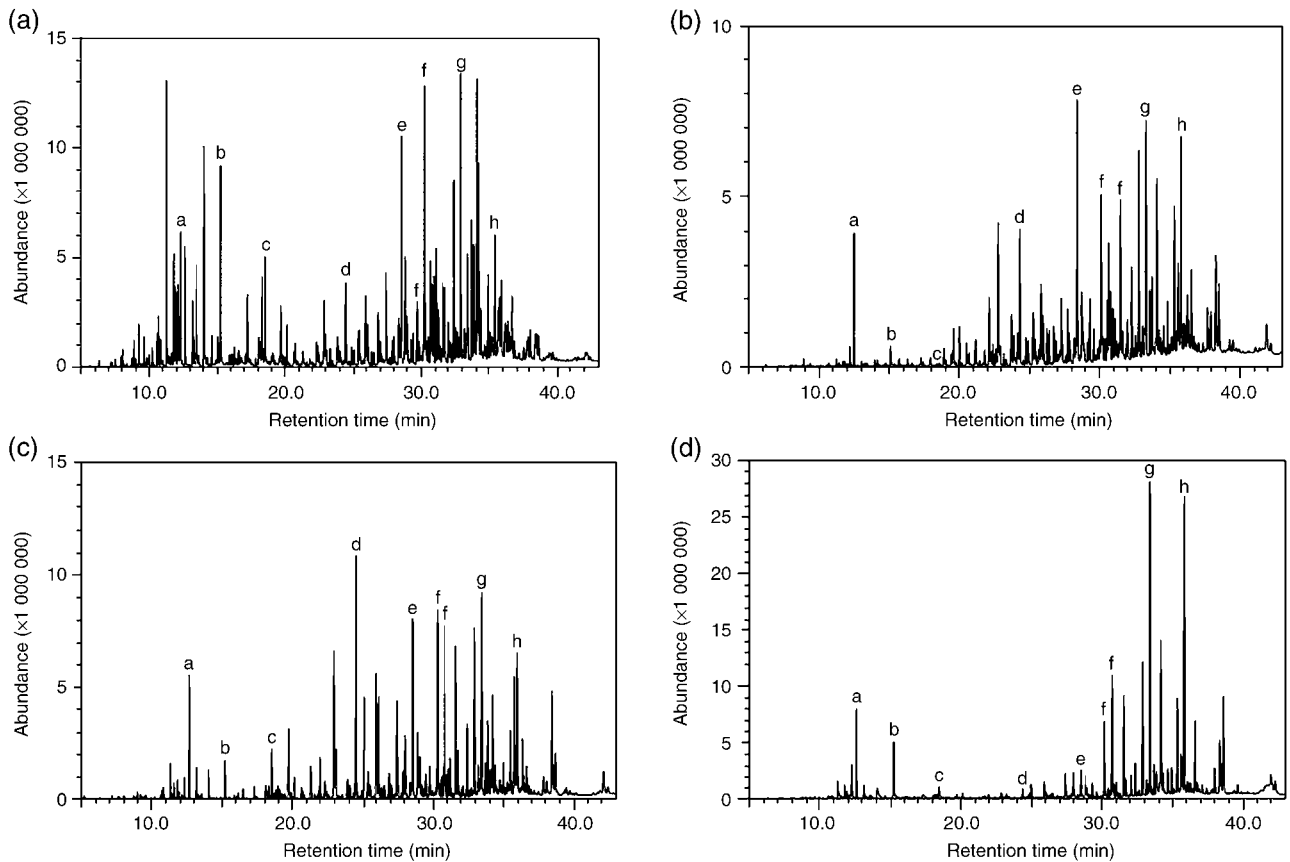


Fig. 2 Chromatograms of the scrotal secretions of two male ring-tailed lemurs. Top row: an outbred male ($H_O = 0.73$) during the (a) nonbreeding and (b) breeding seasons. Bottom row: an inbred male ($H_O = 0.21$) during the (c) nonbreeding and (d) breeding seasons. Letters identify the following compounds: a, internal standard (hexachlorobenzene); b, n-hexadecanoic acid; c, octadecanoic acid; d, octanoic acid, hexadecyl ester; e, squalene; f, isomers of tetradecanoic acid, tetradecyl ester; g, tetradecanoic acid, hexadecyl ester; h, hexadecanoic acid, hexadecyl ester.

Neutral heterozygosity and genetic relatedness

As our primary estimate of male genetic quality, we used mean neutral heterozygosity per individual, H_O (number of heterozygous loci/the total number of genotyped loci; Charpentier *et al.* 2008). This intuitive, symmetrical index varies from 0 to 1, for which 0 refers to the most inbred individuals and 1 refers to the most outbred individuals. We genotyped all of the subjects at 9–14 microsatellite loci (mean loci typed \pm sem: 13.68 ± 0.27) and found a wide range of variation in H_O (mean heterozygosity \pm sem: 0.59 ± 0.03 , range: 0.21–0.79). Furthermore, H_O was highly correlated with three other estimates of heterozygosity: standardized heterozygosity, internal relatedness, and homozygosity by loci (for all tests: $R^2 \geq 0.89$) (Aparicio *et al.* 2006).

Heterozygosity affects phenotypes either because it reflects genome-wide inbreeding or because one or more microsatellite loci are physically linked to functional loci. In the former case, the correlation between genetic diversity and chemical diversity should be equivalent across all neutral microsatellite markers. In the latter case, the relationship

will depend mainly on heterozygosity at a single locus (or at a few loci). Therefore, whenever we detected an effect of H_O , we repeated the analyses, dropping one locus at a time from the calculation of genetic diversity to test for a genome-wide vs. local effect (see also Hoffman *et al.* 2004).

Using the software IDENTIX (Belkhir *et al.* 2002), we calculated the following three estimates of genetic relatedness, R , for each dyad of males: identity (R_{ID}) (Mathieu *et al.* 1990), Queller and Goodnight (R_{QG}) (Queller & Goodnight 1989), and Lynch and Ritland (R_{LR}) (Lynch & Ritland 1999). These three estimates of genetic relatedness have different biases depending on a population's genetic composition. To obtain more accurate estimates of the relatedness between our focal subjects, we calculated these three estimates using a larger, multigeneration data set ($n = 73$; Charpentier *et al.* 2008), representing all available *L. catta*, living and deceased, since the inception of the lemur colony. We then retrieved the dyads that involved only the males included in the present study ($n = 171$ dyads). We also estimated relatedness using the partial pedigree (R_{PED}) of the lemur colony (Appendix S1, Supplementary material). To facilitate

interpretation of the analyses, we calculated matrices of genetic distances among all pairs of males by transforming the four estimates of relatedness (R) into estimates of genetic distances (D) using the formula $D_{xy} = 1 - R_{xy}$, where x and y represent the individuals included in the dyads (Belkhir *et al.* 2002).

Statistical analyses

We first tested for seasonal differences (i.e. breeding vs. nonbreeding) in our three indices of chemical diversity using paired t -tests (SAS version 9, UNIVARIATE procedure). We examined differential expression of specific semiochemicals between seasons using randomization tests in PERM (Duchesne *et al.* 2006). These tests do not assume statistical properties, such as symmetry and homoscedasticity, and are appropriate for variables that are ratios. We computed these tests on the most abundant compounds per individual, between seasons, that is, those ($n = 28$ out of 203) that represented at least 1% of the total chromatogram.

Next, for each season, we examined the relationship between an individual's genetic diversity (using H_O) and each index of its chemical diversity using Pearson correlations (SAS version 9, CORR procedure). Lastly, again for each season, we analysed the relationship between the chemical distance between the males in each possible dyad ($n = 171$; using Euclidean matrices) and the genetic distance between the members of the corresponding dyad (using each of the four relatedness estimates). We performed this analysis by computing partial Mantel tests with 2000 data randomizations (FSTAT version 2.9.3.2; Goudet 2001). In these tests, we also considered the following variables: distance in age between the members of each dyad, distance in months between the odourant collection dates for each dyad member, and difference in the housing conditions of each dyad member (scored as 0 for different conditions or 1 for the same condition).

Results

Seasonal variation in semiochemical diversity

We observed seasonal variation in the olfactory signals of *Lemur catta*, evident when comparing the chromatograms of scent secretion samples obtained during the nonbreeding season (Fig. 2a, c) to those obtained during the breeding season (Fig. 2b, d). More specifically, during the breeding season, we observed a significant loss in chemical diversity relative to the nonbreeding season, reflected in all three indices (paired t -tests; richness: $t_{18} = 5.59$, $P < 0.0001$; Shannon: $t_{18} = 3.47$, $P = 0.003$; Simpson: $t_{18} = 2.73$, $P = 0.01$; Fig. 3). Although 18 of 19 subjects showed a decline in richness representing, on average, a 12.5% drop in the number of compounds present, the males did not systematically lose

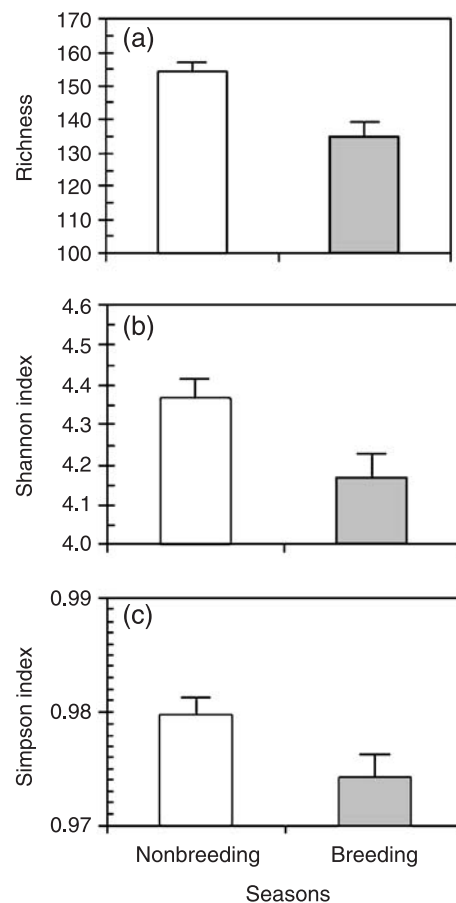


Fig. 3 Mean (\pm SEM) chemical diversity of 19 male ring-tailed lemurs during the nonbreeding and breeding seasons. Chemical diversity is represented by (a) richness (b) Shannon index, and (c) Simpson index.

the same compounds. For instance, across all breeding season samples, 131 different compounds were lost in at least one male, but no single compound was lost by all males. In addition, the relative abundance of cholestanol was significantly reduced in the breeding season (Table S1, Supplementary material). Concomitantly, however, males augmented the relative abundance of three fatty acid esters (Table S1), which likely contributed to the lower Shannon and Simpson indices for this season.

Relation between individual semiochemical diversity and individual genetic diversity

The chemical diversity of male semiochemical profiles corresponded to their genetic quality or diversity, as revealed by H_O , but only seasonally. Neutral heterozygosity did not correlate with any index of chemical diversity during the nonbreeding season ($n = 19$; richness: $R^2 = 0.03$, $P = 0.45$; Shannon: $R^2 = 0$, $P = 0.88$; Simpson: $R^2 = 0$, $P = 0.93$; Fig. 4a). By contrast, H_O significantly and positively correlated with each index of chemical diversity during the breeding

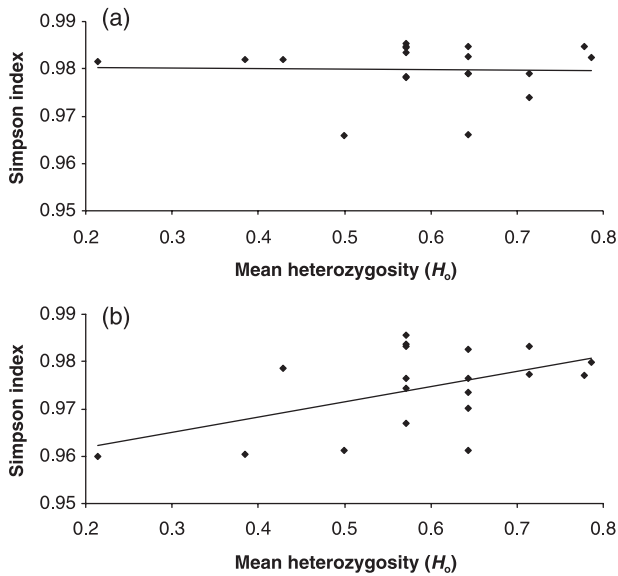


Fig. 4 Relationship between genetic diversity (mean neutral heterozygosity per individual) and chemical diversity (based on the Simpson index) in male ring-tailed lemurs, during the (a) nonbreeding and (b) breeding seasons. A greater Simpson Index indicates increased diversity in semiochemical compounds.

season ($n = 19$; richness: $R^2 = 0.24$, $P = 0.03$; Shannon: $R^2 = 0.22$, $P = 0.04$; Simpson: $R^2 = 0.27$, $P = 0.02$; Fig. 4b). Therefore, the seasonal decline in chemical diversity that we had observed overall (Figs 2 and 3) was more pronounced in the more homozygous (or inbred) males than in the more heterozygous (or outbred) males.

These findings were not attributable to other socio-demographic variables, including the male's age, the month in which the scent sample was collected, and the housing condition (Appendix S2, Supplementary material). Moreover, three other estimates of genetic diversity (i.e. standardized heterozygosity, internal relatedness, and homozygosity by loci) showed patterns that generally were consistent with those obtained using H_O (Appendix S3 and Table S2, Supplementary material). Lastly, when we repeated the analyses, dropping one locus at a time from the calculation of genetic diversity, our results maintained: we failed to find locus-specific effects on chemical diversity. Therefore, H_O proved to be a good estimate of genome-wide inbreeding (Appendix S4 and Table S3, Supplementary material; Charpentier *et al.* 2008).

Relation between semiochemical distances and genetic distances between males

The semiochemical distances between male lemurs also predicted their genetic relatedness, but again the relationship was evident only seasonally. During the nonbreeding season, no estimate of genetic distance correlated with

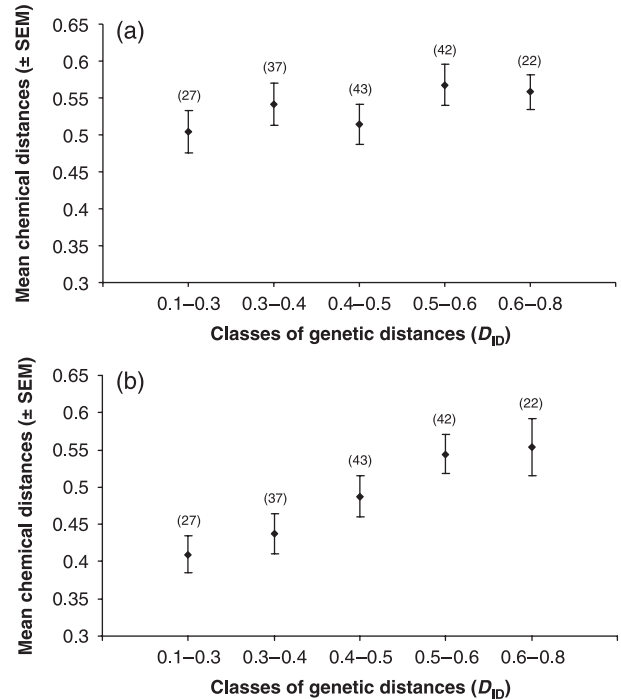


Fig. 5 Relationship between classes of genetic distances (D_{ID}) and mean chemical distances (relative Euclidean) in 171 dyads between 19 ring-tailed lemurs, during the (a) nonbreeding and (b) breeding seasons. The analyses were conducted on the 171 dyads using partial Mantel tests; however, for the ease of representation, we illustrate these relationships using mean chemical distances per class of genetic distances. The latter were defined by equilibrating their sample sizes (given in parentheses).

chemical distance (D_{ID} : partial $R^2 = 0.01$, $P = 0.17$; Fig. 5a; D_{QG} : partial $R^2 = 0$, $P = 0.88$; D_{LR} : partial $R^2 = 0$, $P = 0.88$; D_{PED} : partial $R^2 = 0$, $P = 0.96$). During the breeding season, however, D_{ID} , D_{QG} , and D_{LR} each correlated with chemical distance (partial $R^2 = 0.11$, $P < 0.001$; Fig. 5b; partial $R^2 = 0.04$, $P = 0.01$; partial $R^2 = 0.02$, $P = 0.05$, respectively), whereas D_{PED} did not (partial $R^2 = 0$, $P = 0.45$). Therefore, during the breeding season, chemical distance increased with increasing genetic distance between dyad members. The absence of a breeding-season effect for D_{PED} , despite its correlation with the other measures of genetic distance ($R^2 \geq 0.40$, $P < 0.0001$ for all pairwise comparisons), may indicate that semiochemicals signal overall genetic distance, rather than kinship per se. Finally, none of the three other covariables defined above correlated with chemical distances during both seasons (e.g. for the estimate identity during the breeding season, age difference: $R^2 = 0$, $P = 0.75$; distance in months: $R^2 = 0$, $P = 0.41$; housing differences: $R^2 = 0$, $P = 0.89$).

Discussion

We provide the first evidence, in any species, that semiochemical profiles within and between males indeed reflect

two types of genetic information — individual heterozygosity and genetic distance — but in a seasonally dependent fashion. Olfactory information about the genetic quality of and genetic distance between male ring-tailed lemurs becomes available in scrotal secretions only during the breeding season, when both male–male competition and female mate choice should be crucial. More specifically, deciphering the scrotal scent signals of male lemurs may resolve an important paradox in sexual selection theory, as these signals could convey both good genes *and* compatibility — information that would be required for a female to choose *her* best mate.

In various species, the breeding season represents a period of change in secretory glands and their olfactory signals, a phenomenon typically attributed to the up-regulation of sex steroids (Ebling 1977; Albone 1984; Müller-Schwarze 2006). Thus, one might have predicted an increase in lemur semiochemical diversity with a seasonal increase in androgen production. Although we observed an increase in some of the most abundant compounds (e.g. three fatty acid esters), the breeding season was more generally characterized by a loss of compound diversity. Aspects of *Lemur catta* life history may shed some light on this potentially unexpected finding.

Importantly, the breeding season represents a stressful period for male lemurs, as they compete intensively over brief and limited reproductive opportunities: they receive aggression both from other male suitors and, unusually, from the dominant females rejecting their advances (Drea 2007). Coincident with testicular recrudescence, dramatically increased androgen secretion, and fighting at this time (Drea 2007), male faecal glucocorticoid concentrations increase ~1.5 times over nonbreeding season values (A. Starling, C. Fitzpatrick, E.S. Scordato & C.M. Drea, Duke University, unpublished data). Stress- or seasonally induced changes in chemical expression have been documented in other species (Von Schantz *et al.* 1999; Soini *et al.* 2007). Like visual or acoustic signals (Von Schantz *et al.* 1999), olfactory signals may be altered by stress-induced production of, for example, reactive metabolites or free radicals. The breeding-season drop in chemical diversity therefore could signal that males are challenged or unable to sustain the production or expression of complex odourants during stressful periods.

As chemical production can be costly (Johansson & Jones 2007), the inbred male lemurs, in particular, may have been less able to express complex semiochemicals during the breeding season, potentially through altered lipid metabolism or through a stress-induced drop in protein production (although the contribution of nonvolatile compounds requires elucidation). We previously documented inbreeding depression in this lemur colony: notably, inbred animals are more compromised on several health parameters and die earlier from diseases than do outbred animals (Charpentier *et al.* 2008). Thus, similar to differences in scent-marking behaviour that alter the olfactory landscape and are costly to maintain

(Rich & Hurst 1999), chemically based visual cues or adornments can honestly advertise male quality (Faivre *et al.* 2003; López *et al.* 2006; McGlothlin *et al.* 2008).

Whatever the mechanism responsible for the decline in chemical diversity during the critical breeding season, we show that more heterozygous lemurs better maintain complex olfactory signals than do less heterozygous lemurs. Therefore, odourant profiles represent condition-dependent signals that reflect not only variation at MHC genes, as has been previously described in mice (Willse *et al.* 2005; Willse *et al.* 2006), but an individual's overall genetic variation. Our latter finding suggests that potentially greater diversity exists in the olfactory indicators of male quality. Based on the limited frequency of olfactory investigation of male scent marks by female lemurs, we previously suggested that behavioural cues may outweigh olfactory cues in female mate choice (Scordato & Drea 2007); however, whereas the ever changing dominance relations between reproductively active males may require continual olfactory signalling and reassessment by male competitors, a female need investigate a male's scrotal mark maybe only once in the breeding season to acquire information about his genetic diversity. Therefore, male odours would appear to be particularly relevant, to both sexes, during the breeding season.

Results from behavioural bioassays frequently implicate an olfactory mechanism of kin discrimination (Penn 2002; Mateo 2003), but in only one set of studies have researchers reported a correspondence between kinship and semiochemistry (Sun & Müller-Schwarze 1998a, b). Here, we provide evidence that the semiochemical distances between pairs of males also reflect the males' genetic distances. Kin recognition presumably functions by self-referent or known-kin matching, by which animals avoid breeding with or preferentially direct aid to individuals that share similar scent signatures to their own or to their known kin (Mateo 2003). Our findings are consistent with that mechanism; however, because the olfactory signal of genetic distance between male lemurs appears only during the breeding season, we suggest that their scent marks might serve seasonally dependent functions, including signalling genetic compatibility to breeding females or avoiding confrontation with potential male relatives.

Additionally, we suggest that other olfactory-guided behaviour could function by a comparably simple rule of thumb to self-referent matching. For instance, females could reliably identify genetically diverse mates by selecting those that express the most chemically complex signals, either year round or solely during the breeding season. If these same males prove to be the most competent competitors, their semiochemical complexity also could identify them as males to avoid in intrasexual contest. Therefore, scent marking by male ring-tailed lemurs could be viewed as a sexually selected trait. By combining chemical and genetic

analyses, we have established a novel analytical approach that promises to have broad relevance for revealing the mechanisms by which olfactory cues influence mate choice, intrasexual competition, and kin discrimination.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1 Calculation of D_{PED}

Appendix S2 Multivariate analysis of chemical and genetic diversity

Appendix S3 Relating chemical diversity to mean individual heterozygosity, standardized heterozygosity, internal relatedness and homozygosity by loci

Appendix S4 Testing if all loci contribute equally to variation in chemical diversity indices

Table S1 Seasonal differences in the expression of semiochemicals representing at least 1% of chromatograms ($n = 28$)

Table S2 Pearson correlations between H_O , H_S , IR, and H_L and the three indices of chemical diversity across seasons

Table S3 Pearson correlations between heterozygosity at $N - 1$ loci and chemical diversity indices during the breeding season

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