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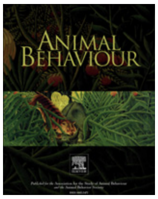

# Message ‘scent’: lemurs detect the genetic relatedness and quality of conspecifics via olfactory cues

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To enhance the fitness benefits of social and sexual interaction, animals should be able to decipher information about the genetic makeup of conspecifics. The use of relative criteria to estimate genetic relatedness could facilitate nepotism or inbreeding avoidance, and the use of absolute criteria to estimate genetic quality could help identify the fittest competitor or the best mate. For animals to process trade-offs between relatedness and quality, however, both relative and absolute genetic information must be concurrently available and detectable by conspecifics. Although there is increasing evidence to suggest that animals make genetically informed decisions about their partners, and may even process trade-offs, we understand relatively little about the sensory mechanisms informing these decisions. In previous analyses of the olfactory signals of ringtailed lemurs, *Lemur catta*, we showed that both scrotal and labial secretions seasonally encode chemical information about (1) pairwise genetic relatedness, within and between the sexes, and (2) individual heterozygosity. Here, using a signaller–receiver paradigm, we conducted behavioural bioassays to test whether male and female lemurs are sensitive to these olfactory sources of genetic information in unfamiliar conspecifics. As the lemurs discriminated conspecific glandular secretions by pairwise relatedness and individual heterozygosity, volatile olfactory signals can be used by both sexes to concurrently process relative and absolute genetic information about conspecifics. Beyond supporting an olfactory mechanism of kin discrimination and mate choice in a primate, we suggest that animals could use olfactory processing to trade off between selection for the most compatible partner versus the most genetically diverse partner.

Genetic relatedness is a major determinant of social and sexual relationships in animals (Armitage 1987; Blouin & Blouin 1988; Pusey & Wolf 1996; Andersson & Simmons 2006). Genetic compatibility between social or sexual partners can significantly impact an individual’s direct and indirect fitness, with benefits of appropriate partner selection being evidenced, for instance, by the preferential treatment of relatives (Holmes 2001), the avoidance of consanguineous mating, or the production of offspring with specific genotypes (Pusey & Wolf 1996; Tregenza & Wedell 2000). The influence of an individual’s genetic quality on its social and sexual interactions is likewise crucial, as evidenced by the competitive superiority of genetically diverse individuals (Meagher et al. 2000; Tiira et al. 2003, 2006), the social isolation of less genetically diverse individuals (Charpentier et al. 2008a), or the reproductive advantage of the ‘best’ mate possessing ‘good genes’ (Neff & Pitcher 2005; Andersson & Simmons 2006). Because an animal’s appropriateness as a social partner or mate may be context dependent, it stands to reason that animals should consider a range of genetic information before selecting a social or sexual partner. These considerations could potentially lead to a trade-off between selection based on a relative criterion (e.g. genetic relatedness or compatibility between a signaller and receiver) versus selection based on an absolute criterion (e.g. intrinsic genetic quality of a signaller; Mays & Hill 2004). Indeed, there is recent theoretical (Colegrave et al. 2002) and empirical (Roberts & Gosling 2003; Hoffman et al. 2007; Schwensow et al. 2008; Eizaguirre et al. 2009) evidence to suggest that such trade-offs occur, specifically in the context of mate choice (Neff & Pitcher 2005). Nevertheless, we understand little about the sensory mechanisms by which animals process such trade-offs or about the potential broader applicability of trade-offs to other types of social relationships.

In various species tested behaviourally, discriminatory responses to a selection of conspecific scents suggest that olfactory cues encode information about genetic relatedness and compatibility

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between potential mates (reviewed in: Penn 2002; Todrank & Heth 2003; Johnston 2008) or about estimates of genetic quality (Willse et al. 2006; Johansson & Jones 2007; Thom et al. 2008), information that is readily used by prospective mates (Wedekind et al. 1995; Penn & Potts 1999; Penn 2002; Olsson et al. 2003; Milinski et al. 2005; Parrott et al. 2007; Eizaguirre et al. 2009; Ilmonen et al. 2009). Rarely, however, have the two types of genetic information been concurrently linked to olfactory information available in the same species or have animals been shown to detect both types of genetic information (but see Roberts & Gosling 2003; Hoffman et al. 2007; Schwensow et al. 2008; Eizaguirre et al. 2009).

In our own studies of ringtailed lemurs, *Lemur catta*, a highly sociable strepsirrhine primate, we first used a chemical approach, based on gas chromatography and mass spectrometry (GCMS), rather than a behavioural approach, to link olfactory cues to genetic variability. We have shown that both relative and absolute genetic information are available within the same olfactory signal, particularly during the highly competitive breeding season. Notably, the semiochemicals expressed in male and female genital secretions encode information about pairwise genetic relatedness, seasonally within the sexes and year round between the sexes (Charpentier et al. 2008b; Boulet et al. 2009). Additionally, male (Charpentier et al. 2008b) and female (Boulet et al., in press) odorants seasonally encode information about individual genome-wide heterozygosity. Individuals with high mean heterozygosity generally possess phenotypically superior traits that are thought to confer direct benefits to their partners (Partridge 1983). In addition, an individual's heterozygosity can confer indirect benefits to its mate: Although individuals cannot pass onto offspring their heterozygosity at specific loci (Mays & Hill 2004), heterozygous individuals tend to sire more heterozygous offspring when allelic frequencies are asymmetric (Mitton et al. 1993). Because heterozygosity in our study population correlates with various health parameters and predicts survivorship (Charpentier et al. 2008c), olfactory cues appear to be honest indicators of individual quality (Charpentier et al. 2008b; Boulet et al., in press).

Here, we used behavioural bioassays to test whether male and female lemurs are sensitive to these olfactory sources of genetic information about their conspecifics. We used an experimental, signaller–receiver paradigm in which we presented single recipients with pairs of odorants derived from two unfamiliar, conspecific scent donors. For each recipient, we used available genetic information to select two odorant donors that had a minimum difference in heterozygosity between them of 0.2. Thus, the signals varied based on absolute criteria. We also selected the odorant donors so that they varied in degree of pairwise genetic relatedness relative to the recipient. Thus, the signals also varied based on relative criteria. Trials involving intersexual investigation (i.e. males presented with female odorants or 'M vs FF' trials; females presented with male odorants or 'F vs MM' trials) are most relevant for understanding kin or quality discrimination in the context of inbreeding avoidance and mate choice, whereas trials involving intrasexual investigation (i.e. males presented with male odorants, 'M vs MM' trials; females presented with female odorants, 'F vs FF' trials) are most relevant for understanding kin or quality discrimination in the context of nepotistic or competitive behaviour.

## METHODS

### Subjects

The study involved 33 reproductively intact, adult ringtailed lemurs, including 17 males and 16 females, aged 3–25 years. Of these, 20 (9 M, 11 F) served as odorant recipients, and 32 (16 M, 16 F) served as odorant donors. Pregnant females were excluded from the study. Recipients were matched with 'unfamiliar' donors, defined as animals that had never lived in the same social group with the recipient (as in Scordato & Drea 2007). The lemurs were all captive born and housed under varying social and spatial conditions (Scordato & Drea 2007; Scordato et al. 2007) at the Duke Lemur Center (DLC; Durham, NC, U.S.A.). The DLC is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. Animal care met with institutional guidelines and was in accordance with regulations of the United States Department of Agriculture. All research protocols were approved by the Institutional Animal Care and Use Committee of Duke University (protocol numbers A245-03-07 and A232-06-07).

### Odorant Sample Collection

We collected odorant samples monthly from the scrotal and the labial glands of awake, manually restrained animals. Odorant collection spanned from August 2004 through May 2008, encompassing periods representing both the breeding and nonbreeding seasons. This extended collection period allowed us to increase our pool of odorants, which otherwise would have been limited by our criterion for 'unfamiliar' donors. As in Scordato et al. (2007), we used prewashed cotton swabs and sterile forceps for collection and placed scented swabs in solvent-washed chromatography vials. We stored the samples at −80 °C until their use in behavioural bioassays. As previously demonstrated, freezer storage does not alter the semiochemistry of odorant samples (Scordato et al. 2007; Lenochova et al. 2008).

### Estimates of Relatedness and Heterozygosity

To estimate the genetic relatedness between recipient–donor pairs and donor genome-wide heterozygosity ($H_O$), we genotyped our subjects at 14 microsatellite loci (as in Boulet et al. 2009; for details on the methods and the loci used see Charpentier et al. 2008b). We calculated estimates of genetic relatedness between our 33 subjects using a larger, historical genetic data set available for DLC colony members ($N = 81$ individuals genotyped at 11–14 microsatellite loci, as in Boulet et al. 2009). Using the software Identix (Belkhir et al. 2002), we calculated the following three estimates of genetic relatedness, $R$, for each dyad of individuals: identity, $R_{ID}$ (Mathieu et al. 1990), Queller & Goodnight, $R_{QG}$ (Queller & Goodnight 1989) and Lynch & Ritland, $R_{LR}$ (Lynch & Ritland 1999) (also see Charpentier et al. 2008b). These three estimates of genetic relatedness have different biases depending on the distribution of allele frequencies and the percentage of related individuals present in the study population (Van de Casteele et al. 2001; Belkhir et al. 2002), but produced comparable results for our study population (for a more comprehensive discussion of these estimates, see Charpentier et al. 2008b).

We calculated each individual's $H_O$ by dividing the number of heterozygous loci by the number of genotyped loci. The DLC lemur population represents reasonable variation in $H_O$, with our 32 odorant donors ranging in $H_O$ from 0.21 (relatively 'inbred') to 0.82 (relatively 'outbred') on a scale of 0–1 (mean ± SE = 0.57 ± 0.02). To control for variation in genetic diversity presented to each recipient, we identified pairs of unfamiliar donors that differed by at least 0.2 in their heterozygosity.

Before conducting the statistical analyses, we first verified that the estimates of relatedness and $H_O$ were not correlated in any of our four data sets (M vs FF, M vs MM, F vs MM, F vs FF) and selected the indexes that were the least correlated with $H_O$ for any given model. Initially, for the M vs FF model, all three indexes correlated significantly with $H_O$. To resolve this issue, we used a diagnostic measure of influence, leverage coefficients (Sokal & Rohlf 1995), to identify the triads (i.e. the unique trio formed by a given recipient and pair of donors) that were driving this correlation. We then removed the culprit triads ($N = 2$) from our data. This adjustment involved removing four behavioural bioassays, as each triad was tested during the breeding and nonbreeding seasons. Lastly, we used $R_{LR}$ for all of the models, save one. The exception involved M vs MM trials, for which we instead used $R_{QG}$. As noted above, we used these two estimates of relatedness because they correlated least with $H_O$ for their respective models.

### *Behavioural Bioassays*

The behavioural bioassays performed in this study followed previously published procedures (Scordato & Drea 2007; for footage of a male lemur's behavioural response to female odorants, see electronic Supplementary Material, Video S1, Fig. S1). Briefly, we temporarily isolated the recipient animal from its social companions by allowing it to enter one of its habitual enclosures, where, after a 10 min habituation period, we tested its response to three new and freshly scented dowels, placed 25 cm apart along one side of the enclosure. We rubbed each outer 'test' dowel with one of the donors' secretions, and rubbed the centre 'control' dowel with a clean cotton swab. We randomized placement of the paired odorants between the test dowel positions so that the odorant from the more outbred donor of each dyad alternated between the left and right dowels. We recorded the behavioural response to the three dowels during 15 min trials.

We conducted 150 behavioural bioassays, 73 during the breeding season (November–December 2007) and 77 during the nonbreeding season (April–June 2008), with 1–37 trials per recipient. Most (88.7%) of the triads were represented during both seasons. We matched the season of odorant sample collection to the season of behavioural testing and we matched paired samples by their month of collection for 49.7% trials. Of the 150 trials, two replicate trials were conducted using the same four triads. For these four triads, we used the average of the recipient's response across their replicate trials in our data analysis. The number of 'adjusted' trials ($N = 142$), which accounted for collinearity between $R$ and $H_O$ (see above) and triad duplication, varied across type of bioassay: $N = 49$ for M vs FF trials; $N = 46$ for M vs MM trials; $N = 27$ for F vs MM trials; and $N = 20$ for F vs FF trials. In these 142 trials, the 32 odorant donors contributed 1–24 samples (mean ± SE = 8.9 ± 1.2).

We videotaped the trials, as previously described (Scordato & Drea 2007) and digitized the video footage using Ulead VideoStudio 11 (Corel). Two observers blind to any genetic information about the subjects scored the trials. We analysed the following behavioural responses in both sexes (for more information, see Scordato & Drea 2007): time spent in proximity (within 12 cm) of a given dowel while showing no other behavioural response, and time spent sniffing the dowel (with the nose held at or within 2 cm of the placement of the odorant). We analysed the time spent licking the dowel in males only, as licking was virtually absent in females. Sniffing reflects olfactory investigation of the volatile fraction of the scent signal, whereas licking reflects gustatory investigation of the nonvolatile fraction. Lastly, we analysed sexually differentiated patterns of scent-marking behaviour. In males, we measured the combined number of wrist marks and shoulder rubs only (see Figure 1 in Charpentier et al. 2008b) as, in the present study, males showed little to no genital (or scrotal) marking. In females, we measured the number of genital (or labial) marks performed. All behavioural categories were mutually exclusive. We calculated interobserver reliability using indexes of concordance (Martin & Bateson 1993) as described in Scordato & Drea (2007), which were as follows: proximity = 88%, sniff = 94%, lick = 100%, genital mark = 100%, and wrist/shoulder marking = 90%.

### *Statistical Analyses*

To validate differential response allocations to the test versus control dowels, we performed Wilcoxon signed-ranks tests (Univariate procedure, SAS v9, SAS Institute, Cary, NC, U.S.A.). For each behaviour, we compared the average duration or frequency directed to the two test dowels to the duration or frequency directed to the control dowel. For 13 of the 14 (92.9%) interaction types (proximity, sniffing, licking and marking displayed by males towards male and female odorants, as well as proximity, sniffing and marking displayed by females towards male and female odorants), the recipients directed significantly more responses ($P < 0.05$) to the scented dowels.

For each type of interaction, we calculated the difference in behavioural responses to the two test dowels by subtracting the total duration or frequency of behaviour directed to the left test dowel from the total duration or frequency of behaviour directed to the right test dowel. We likewise calculated the difference in pairwise relatedness ($D_{LR}$ and $D_{QG}$) between the two recipient–donor pairs involved in each trial. Lastly, we calculated the difference in $H_O$ ($D_{Ho}$) between the two odorant donors involved in each trial. The resulting absolute mean differences for relatedness ($D_{RL}$) and heterozygosity ($D_{Ho}$) for our four types of bioassays were as follows: M vs FF: $D_{LR} = 0.130$, $D_{Ho} = 0.264$; M vs MM: $D_{QG} = 0.264$, $D_{Ho} = 0.276$; F vs MM: $D_{LR} = 0.184$, $D_{Ho} = 0.316$; F vs FF: $D_{LR} = 0.149$, $D_{Ho} = 0.304$.

Because our data were normally distributed (Shapiro–Wilk tests: $P > 0.05$), we used general linear mixed models (Mixed procedure, SAS v9) to assess the influence of genetic characteristics and season on the lemurs' behavioural responses. To correct for the nonindependence of data points, we considered both recipient identity and donor dyad as random effects. We used a backward model selection procedure to select a set of best-fit explanatory variables. Specifically, we started with all potential explanatory effects (Supplementary Table S1) incorporated into a restricted maximum likelihood model and then sequentially removed the effect with the highest $P$ value from the model, starting with the interaction terms. We repeated these steps until all $P$ values for remaining covariates were less than 0.10. To best illustrate the effect due to the variable of interest, we graphically represent our results using the residuals obtained from the regression analyses.

Because we conducted multiple testing on related behavioural data sets, we performed a binomial test on the overall data set (combining the four categories of trials) to test whether the number of significant genetic effects detected was greater than expected by chance (following Teriokhin et al. 2007). We observed significantly more genetic effects on behavioural responses than expected by chance, whether we considered only statistically significant effects (number of successes: 7; number of trials: 56; threshold: 0.05; $P = 0.02$) or also included marginal effects (number of successes: 11; number of trials: 56; threshold: 0.05; $P < 0.0001$). These results suggest that the genetic effects observed are biologically meaningful.

## RESULTS

### *Response of Male Recipients to Conspecific Female or Male Odorants*

Males biased their behaviour according to the genetic characteristics of conspecifics. For instance, in M vs FF trials ($N = 49$; Supplementary Table S1), male recipients spent significantly more time licking the scent secretions of females to whom they were less closely related (main effect: $F_{1,47} = 6.57$, $P = 0.02$, $R^2 = 6.9\%$). This behavioural response was influenced by season, however (interaction effect: $F_{1,47} = 9.98$, $P = 0.004$; Fig. 1), as male recipients responded most strongly to the odour of less related females during the breeding season ($R^2 = 40\%$; Fig. 1a), but showed no discriminatory response during the nonbreeding season ($R^2 = 1.6\%$; Fig. 1b).

With respect to genetic quality, we found a marginal main effect: male recipients tended to spend more time in proximity to

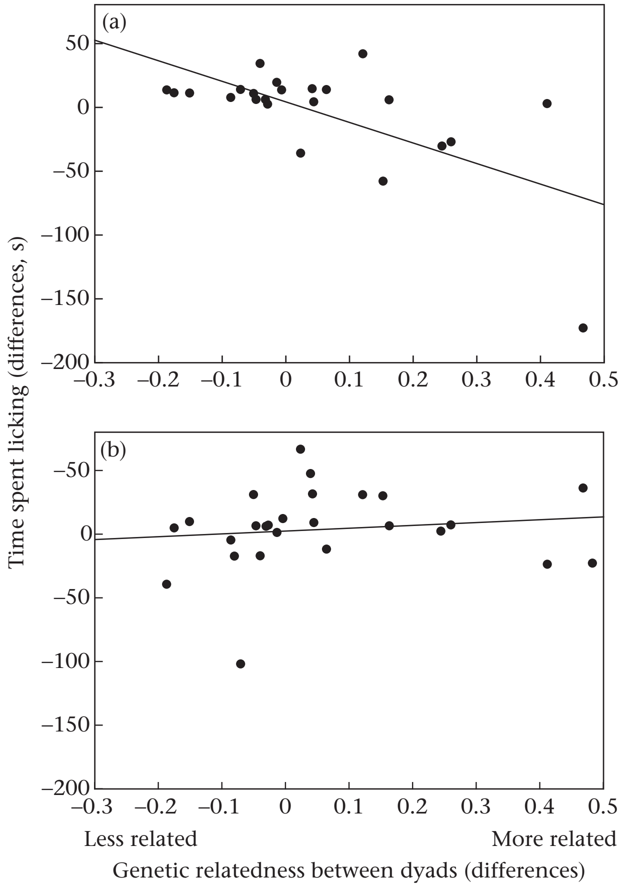

**Figure 1.** Licking responses of male ringtailed lemurs to the odorants of varyingly related conspecific females in the (a) breeding and (b) nonbreeding seasons. Data points represent residuals obtained from a regression that included the random effects considered in the analysis. Relatedness values represent differences between the recipient's pairwise relatedness to each of the two female odorant donors (see Methods). Licking responses represent differences between the time spent licking both odorants (see Methods).

scent derived from less heterozygous females (or, conversely, tended to avoid the scent of more heterozygous females: $F_{1,47} = 3.95$, $P = 0.06$, $R^2 = 4.9\%$).

In M vs MM trials ($N = 46$; Supplementary Table S2), male recipients showed significant seasonal differences in their manner of responding to the odorants of differentially related males (interaction effect for proximity: $F_{1,44} = 4.61$, $P = 0.04$; Fig. 2a, b; interaction effect for sniffing: $F_{1,44} = 4.28$, $P = 0.05$; Fig. 2c, d); however, the seasonal patterns that emerged differed by behavioural category. For instance, during the breeding season, male recipients showed a weakly negative correlation between their time in proximity to odorants and their relatedness to the donor ($R^2 = 11\%$; Fig. 2a), but a weakly positive correlation between their time spent sniffing odorants and their relatedness to the donor ($R^2 = 12.1\%$; Fig. 2c). Broadly speaking, males in the breeding season associated more with the odorants of nonrelatives, but investigated more the odorants of relatives. These patterns were reversed during the nonbreeding season (proximity: $R^2 = 9.2\%$; Fig. 2b; sniffing: $R^2 = 7.9\%$; Fig. 2d).

### *Response of Female Recipients to Conspecific Male or Female Odorants*

Females also biased their behaviour according to the genetic characteristics of conspecifics. For instance, in F vs MM trials ($N = 27$; Supplementary Table S3), female recipients spent significantly more time near odorants derived from less related males (main effect: $F_{1,25} = 8.61$, $P = 0.015$, $R^2 = 21.3\%$; Fig. 3a). Although this pattern was stronger during the nonbreeding season ($R^2 = 39.2\%$) than during the breeding season ($R^2 = 5.6\%$), the interaction effect for proximity only approached significance ($F_{1,25} = 3.89$, $P = 0.08$). Conversely, females spent significantly more time sniffing the odorants of more related males (main effect: $F_{1,25} = 5.08$, $P = 0.05$, $R^2 = 8.5\%$; Fig. 3b). Therefore, year round, females associated more with odorants of unrelated males, but investigated more the odorants of relatives.

With respect to genetic quality, we found marginal seasonal modulation of female behaviour based on the heterozygosity of male donors (interaction effect: $F_{1,25} = 4.08$, $P = 0.07$): during the breeding season, females tended to spend more time sniffing odorants derived from less heterozygous males ($R^2 = 7.9\%$), whereas during the nonbreeding season, the reverse pattern emerged ($R^2 = 16.4\%$).

Lastly, in F vs FF trials ($N = 20$; Supplementary Table S4), regardless of season, female recipients spent significantly more time near the odorants derived from more heterozygous females (main effect: $F_{1,18} = 8.15$, $P = 0.02$, $R^2 = 29.9\%$; Fig. 4). Female recipients also tended to scent-mark more in response to odorants derived from more heterozygous females than they did in response to odorants derived from less heterozygous females (main effect: $F_{1,18} = 4.46$, $P = 0.06$, $R^2 = 18.8\%$). Females therefore showed more consistent olfactory interest in 'outbred' female conspecifics.

## DISCUSSION

In the quest to resolve whether animals evaluate conspecifics for 'good fit' or 'good genes' via relative or absolute genetic information, researchers typically examine the transmission of only one type of genetic signal (but see Roberts & Gosling 2003; Hoffman et al. 2007; Schwensow et al. 2008; Eizaguirre et al. 2009). Furthermore, researchers typically employ a proxy 'mate choice' paradigm to assess the female's proclivity for males of varying genetic characteristics (Wedekind et al. 1995; Penn & Potts 1999; Penn 2002; Olsson et al. 2003; Milinski et al. 2005; Parrott et al. 2007; Ilmonen et al. 2009), without addressing the male's genetically informed proclivity for potential female mates. In prior chemical studies using a primate model (Charpentier et al. 2008b; Boulet et al. 2009, in press), we have shown that both sources of genetic information (relative and absolute) are concurrently signalled by members of both sexes via their glandular scent secretions. Here, we complemented those chemical data by showing that males and females are capable of detecting both types of genetic information in the scent marks of conspecifics. Specifically, male ringtailed lemurs are at least capable of detecting their relatedness to male and female conspecifics, and they probably also detect the heterozygosity of females. Female ringtailed lemurs are at least capable of detecting their relatedness to male conspecifics and the heterozygosity of female conspecifics, and they probably also detect the heterozygosity of males. Because olfactory sources of information about genetic relatedness and genetic quality are concurrently available, lemurs (and presumably other vertebrates) may use olfactory cues to process trade-offs between selection for 'good fit' and 'good genes', not only in potential mates, but also in potential social partners.

We observed discriminatory behaviour during the breeding season, as anticipated based on the concurrent seasonal emergence of semiochemical markers of genetic characteristics (Charpentier et al. 2008b; Boulet et al. 2009, in press). By contrast, we had not anticipated comparable discriminatory behaviour during the nonbreeding season, as we had previously observed only modest

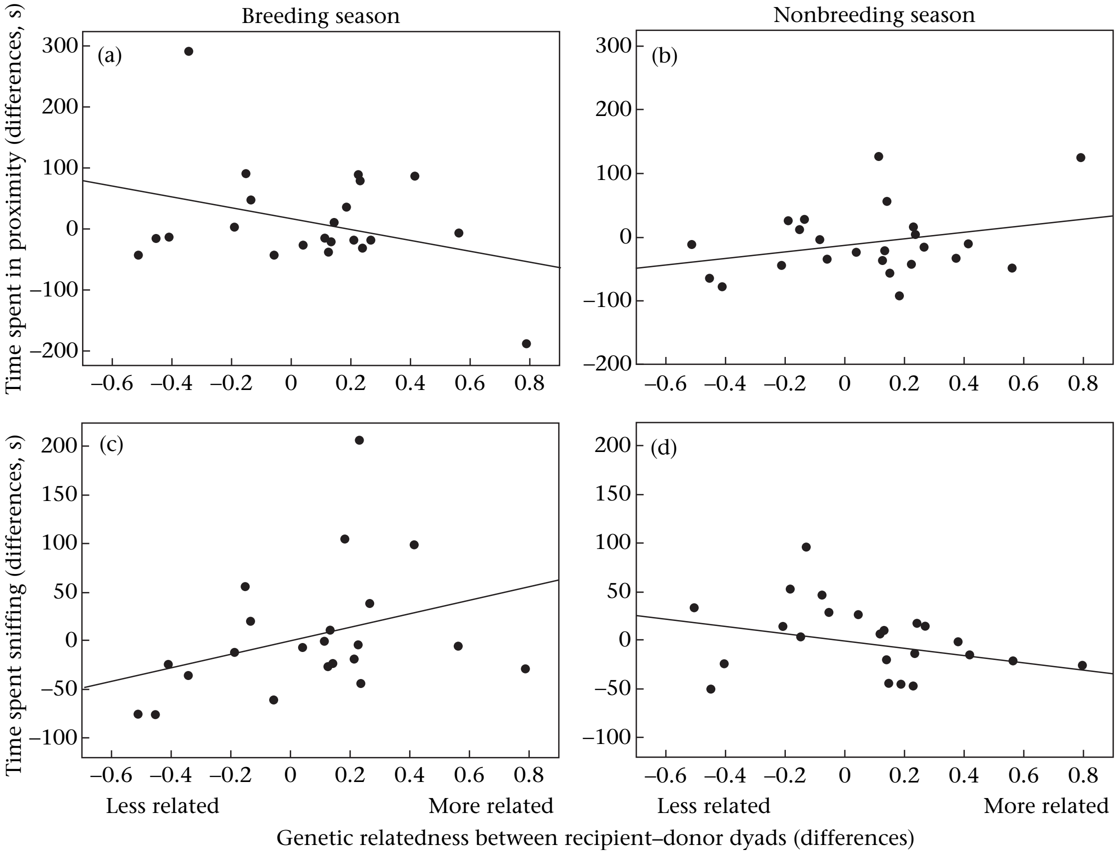

**Figure 2.** Proximity (a, b) and sniffing (c, d) responses of male ringtailed lemurs to the odorants of varyingly related conspecific males in the breeding and nonbreeding seasons. Data points represent residuals obtained from a regression that included the random effects considered in the analysis. Relatedness values represent differences between the recipient's pairwise relatedness to each of the two male odorant donors. Proximity and sniffing responses represent differences between the time spent in proximity to or sniffing both odorants (see Methods).

odour–gene covariance outside of the breeding season (e.g. in mixed-sex pairwise relatedness: $r = 0.12$; Boulet et al. 2009). The behavioural responses in the present study would suggest, therefore, that potentially subtle olfactory markers of genetic characteristics exist year round and, most importantly, that lemurs are sensitive to both strong and subtle cues about genetic variation in conspecifics. This olfactory sensitivity is additionally revealed in the response of females to the scent of other females. Notably, female lemurs detected relatively slight differences (i.e. of 0.20) in neutral heterozygosity. We suspect that stronger effects would have obtained across other signaller–receiver pairs had the choice between two potential partners involved a greater difference in their heterozygosity. Nevertheless, the olfactory acuity evidenced by females suggests that even subtle genetic differences between individuals are likely to be biologically meaningful.

In our behavioural paradigm, we considered a recipient's genetic relatedness to odorant donors as a proxy for genetic compatibility between potential mates and as a measure of kinship between potential social partners. We also considered neutral, genome-wide heterozygosity as a proxy for a donor's overall genetic quality. Evidence of discriminatory behaviour based on these two proxies would suggest that lemurs are capable of using volatile (and, in the case of males, possibly also nonvolatile) olfactory cues to select the most appropriate social and sexual partners.

To the extent that the observed behaviour patterns reflect actual preferences that bear specific socio-ecological relevance, we might conservatively interpret our findings more narrowly. For instance, we found that male lemurs biased important investigatory behaviour specifically during the breeding season: they directed more gustatory investigation towards the odorants of the least related females, and directed more olfactory investigation towards the odorants of the most related males. Assuming that female olfactory cues might inform male mate choice, the male lemurs' intersexual response is consistent with what one might expect for any male vertebrate, particularly within the context of inbreeding avoidance. Although olfactory cues are typically implicated in female mate choice (see for example in humans: Wedekind & Füri 1997; mice: Potts et al. 1991; antechinus: Parrott et al. 2007; sticklebacks: Milinski et al. 2005), there is increasing evidence of potential olfactory-based mate choice in males. For instance, male lizards pair with females that maximize dissimilarity in their offspring at the major histocompatibility complex, MHC (Olsson et al. 2003) and male junglefowl allocate more sperm to MHC-dissimilar females (Gillingham et al. 2009). Our finding that male lemurs discriminate relatedness (and possibly heterozygosity) in the scent of female lemurs is consistent with these vertebrate studies and supports behavioural evidence of male mate choice in our study species (Parga 2006). That discrimination of female scent involved the male lemur licking the odorant is also parsimonious, given the critical role of the mammalian vomeronasal organ in detecting female fertility (Evans & Schilling 1995; Halpern & Martínez-Marcos 2003).

Likewise, the intrasexual response of male lemurs during the breeding season is also consistent with prior suggestions, for other species, that males discriminate relatedness among their potential male competitors to minimize the costs of competing with relatives

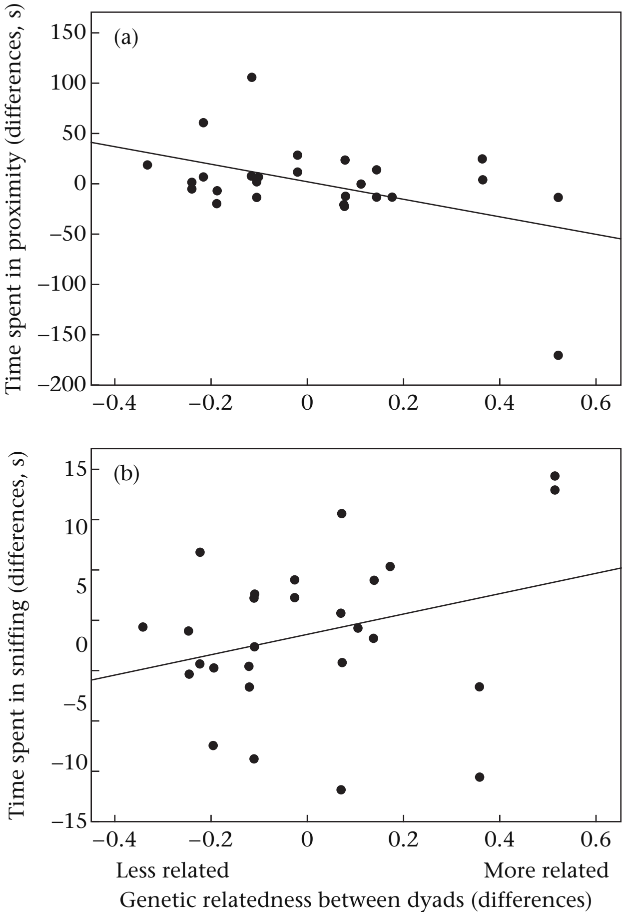

**Figure 3.** Proximity (a) and sniffing (b) responses of female ringtailed lemurs to the odorants of varyingly related conspecific males year round. Data points represent residuals obtained from a regression that included the random effects considered in the analysis (for proximity), or the random effects considered in the analysis along with the other known predictors (for sniffing behaviour; see Supplementary Table S3). Relatedness values represent differences between the recipient's pairwise relatedness to each of the two male odorant donors. Proximity or sniffing responses represent differences between the time spent in proximity to or sniffing both odorants (see Methods).

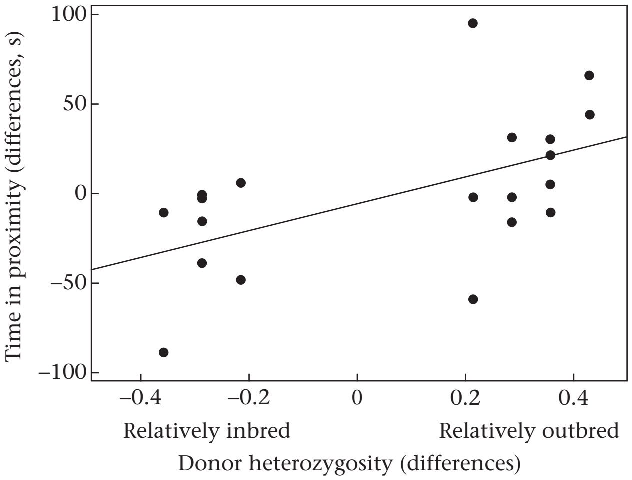

**Figure 4.** Proximity response of female ringtailed lemurs to the odorants of varyingly related conspecific females year round. Data points represent residuals obtained from a regression that included the random effects considered in the analysis. Heterozygosity values represent differences between the heterozygosity of two female odorant donors, and proximity responses represent differences between the time spent in proximity to both odorants (see Methods).

(Trivers 1985; West et al. 2002). During the brief and strictly seasonal breeding period, male ringtailed lemurs engage in exceptionally high rates of intrasexual aggression over access to females and, given the female-dominant social system of this species (Jolly 1966), suffer the additional burden of receiving female aggression (Drea 2007). In this system, minimizing conflict with male relatives while choosing the best or most compatible female mate may help offset the male's reproductive costs. Such may be an example of the potential trade-offs enabled by the concurrent advertisement of relative and absolute genetic information in a given species' scent signals.

Female lemurs also biased their responses to odorants in important ways, mainly year round, but also with an interesting seasonal trend. In particular, during the nonbreeding season, when females would normally be gestating or lactating, their attention to odorants that derived from the most unrelated males was accentuated, whereas throughout the year they biased their investigatory behaviour towards odorants that derived from more closely related males. The differential monitoring of conspecifics, whether relatives or nonrelatives, may relate to the female's changing involvement in nurturing nepotistic relationships or protecting offspring. For instance, during the nonbreeding season, encounters with nonresident males are often aggressive and can result in infanticide (Pereira & Weiss 1991; Hood 1994; Ichino 2005). The risk represented by unfamiliar nonrelatives could well justify concurrently heightened sensitivity to 'foreigner' scent marks by resident members of either sex. By contrast, nepotism could carry benefits year round. Nepotism between unfamiliar relatives has been demonstrated in several anthropoid primate species (Widdig et al. 2001; Silk et al. 2006a, b; Charpentier et al. 2007; reviewed in Widdig 2007). Although odorants have been widely implicated in phenotype matching in rodents (see for example: Mateo 2003), the mechanism for recognizing unfamiliar kin in primates typically has been attributed to social learning. Based on our prior chemical (Charpentier et al. 2008b; Boulet et al. 2009) and present behavioural findings, we suggest that olfactory-based phenotype matching could well occur in lemurs and other primates, including anthropoids, to mediate nepotism and minimize competition between relatives. More broadly, we suggest that social complexity and enhanced encephalization need not obviate olfactory mechanisms of kin recognition and socialization.

Whereas the varying responses of female lemurs could be viewed as trade-offs within the social realm, in other studies, female trade-offs are typically observed in the sexual realm. For instance, researchers have reported that females maximize the genetic diversity of their offspring by choosing both genetically distant partners and partners with 'good genes'. The relative influence of these two genetic factors varies depending on the degree of variability in each male trait. In a subset of these studies, the estimate of good genes is the trait on which females effectuate their choice; however, when the range of choices for the estimate of good genes becomes limited, then genetic distance also matters. For example, in mice, the male's investment in scent marking is a good-genes indicator that consistently predicts female preference; however, when the variability in compatibility among males is relatively large or, conversely, when the variability in marking rates is relatively small, then genetic compatibility also predicts preference (Roberts & Gosling 2003). In fur seals, male heterozygosity strongly predicts female preferences, but heterozygous males become less attractive when they are closely related to the female (Hoffman et al. 2007). In other studies, the most influential variable on female mate choice may be less evident. In dwarf lemurs, for instance, both male genetic diversity (at neutral and MHC loci) and MHC compatibility between mates significantly influence female mate preferences (Schwensow et al. 2008). In sticklebacks, females prefer mates with whom they share an intermediate MHC diversity, as well as males showing a specific MHC haplotype (an estimate of good genes in this study: Eizaguirre et al. 2009). Our findings draw attention to the broader social arena within which such trade-offs may occur.

Lastly, female lemurs also biased their olfactory responses more consistently towards odorants derived from more heterozygous females. Intrasexual female competition presents a continuous challenge, particularly in female-dominant species. The pronounced year-round response (including countermarking) of female recipients to the odorants derived from more heterozygous females may relate to intrasexual competition with high-quality females, particularly with regard to asserting dominance or resource ownership. These findings additionally highlight the importance of female olfactory communication in female-dominant species (Scordato & Drea 2007) and complement chemical evidence of honest olfactory ornamentation in female lemurs (Boulet et al., in press).

By initially deciphering a chemical mechanism for signalling genetic information about pairwise relatedness and individual genetic quality (Charpentier et al. 2008b; Boulet et al. 2009, in press), and subsequently showing that ringtailed lemurs are sensitive to both types of information in the scent marks of conspecifics, we highlight several themes that are broadly relevant to other vertebrate systems. The first is the critical role of olfactory communication in the evolution of complex behavioural interaction. Although well established for asocial taxa (that benefit maximally from broadcast signalling), the role of olfactory cues in mediating the behaviour of socially integrated species only recently has been gaining appreciation (Mateo 2003; Scordato & Drea 2007). Second, with relevance to mechanisms of sexual selection, including intrasexual competition, honest advertisement and mate choice, we draw attention to parallel processes operating via olfactory cues in both sexes. Lastly, we suggest that it is possible for animals to use olfactory signals to evaluate and potentially trade off between relative and absolute genetic criteria when selecting the most appropriate sexual partner or, indeed, the most appropriate social partner. Further studies of the conditions under which males and females might trade off in their choices between conspecifics will help us understand the selective forces acting on the association patterns between animals and provide valuable insight into the operation of kin and sexual selection.

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## Supplementary Material

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## References

**Andersson, M. & Simmons, L. W.** 2006. Sexual selection and mate choice. *Trends in Ecology & Evolution*, **21**, 296–302, doi:10.1016/j.tree.2006.03.015.

**Armitage, K. B.** 1987. Social dynamics of mammals: reproductive success, kinship and individual fitness. *Trends in Ecology & Evolution*, **2**, 279–284.

**Belkhir, K., Castric, V. & Bonhomme, F.** 2002. Identix, a software for relatedness in a population using permutation methods. *Molecular Ecology Notes*, **2**, 611–614.

**Blouin, S. F. & Blouin, M.** 1988. Inbreeding avoidance behaviors. *Trends in Ecology & Evolution*, **3**, 230–233.

**Boulet, M., Charpentier, M. J. E. & Drea, C. M.** 2009. Decoding an olfactory mechanism of kin recognition and inbreeding avoidance in primates. *BMC Evolutionary Biology*, **9**, 281, doi:10.1186/1471-2148-9-281.

**Boulet, M., Crawford, J. C., Charpentier, M. J. E. & Drea, C. M.** In press. Honest olfactory ornamentation in a female-dominant primate. *Journal of Evolutionary Biology*, doi:10.1111/j.1420-9101.2010.02007.x.

**Charpentier, M. J. E., Peignot, P., Hossaert-Mckey, M. & Wickings, E. J.** 2007. Kin discrimination in juvenile mandrills, *Mandrillus sphinx*. *Animal Behaviour*, **73**, 37–45, doi:10.1016/j.anbehav.2006.02.026.

**Charpentier, M. J. E., Prugnolle, F., Gimenez, O. & Widdig, A.** 2008a. Genetic heterozygosity and sociality in a primate species. *Behavioral Genetics*, **38**, 151–158, doi:10.1007/s10519-008-9191-6.

**Charpentier, M. J. E., Boulet, M. & Drea, C. M.** 2008b. Smelling right: the scent of male lemurs advertises genetic quality and relatedness. *Molecular Ecology*, **17**, 3225–3233, doi:10.1111/j.1365-294X.2008.03831.x.

**Charpentier, M. J. E., Williams, C. V. & Drea, C. M.** 2008c. Inbreeding depression in ring-tailed lemurs (*Lemur catta*): genetic diversity predicts parasitism, immunocompetence, and survivorship. *Conservation Genetics*, **9**, 1605–1615, doi:10.1007/s10592-007-9499-4.

**Colegrave, N., Kotiaho, J. S. & Tomkins, J. L.** 2002. Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. *Evolutionary Ecology Research*, **4**, 911–917.

**Drea, C. M.** 2007. Sex and seasonal differences in aggression and steroid secretion in *Lemur catta*: are socially dominant females hormonally 'masculinized'? *Hormones and Behavior*, **51**, 555–567, doi:10.1016/j.yhbeh.2007.02.006.

**Eizaguirre, C., Yeates, S. E., Lenz, T. L., Kalbe, M. & Milinski, M.** 2009. MHC-based mate choice combines good genes and maintenance of MHC polymorphism. *Molecular Ecology*, **18**, 3316–3329, doi:10.1111/j.1365-294X.2009.04243.x.

**Evans, C. & Schilling, A.** 1995. The accessory (vomeronasal) chemoreceptor system in some prosimians. In: *Creatures of the Dark. The Nocturnal Prosimians* (Ed. by L. Alterman, G. A. Doyle & M. K. Izard), pp. 393–411. New York: Plenum.

**Gillingham, M. A. F., Richardson, D. S., Lovlie, H., Moynihan, A., Worley, K. & Pizzari, T.** 2009. Cryptic preference for MHC-dissimilar females in male red junglefowl, *Gallus gallus*. *Proceedings of the Royal Society B*, **276**, 1083–1092, doi:10.1098/rspb.2008.1549.

**Halpern, M. & Martínez-Marcos, A.** 2003. Structure and function of the vomeronasal system: an update. *Progress in Neurobiology*, **70**, 245–318, doi:10.1016/S0301-0082(03)00103-5.

**Hoffman, J. I., Forcada, J., Trathan, P. N. & Amos, W.** 2007. Female fur seals show active choice for males that are heterozygous and unrelated. *Nature*, **445**, 912–914, doi:10.1038/nature05558.

**Holmes, W. G.** 2001. The development and function of nepotism. Why kinship matters in social relationships. In: *Developmental Psychobiology, Developmental Neurobiology and Behavioral Ecology: Mechanisms and Early Principles* (Ed. by E. M. Blass), pp. 281–316. Cambridge: Cambridge University Press.

**Hood, L. C.** 1994. Infanticide among ringtailed lemurs (*Lemur catta*) at Berenty Reserve, Madagascar. *American Journal of Primatology*, **33**, 65–69.

**Ichino, S.** 2005. Attacks on a wild infant ring-tailed lemur (*Lemur catta*) by immigrant males at Berenty, Madagascar: interpreting infanticide by males. *American Journal of Primatology*, **67**, 267–272, doi:10.1002/ajp.20183.

**Ilmonen, P., Stundner, G., Thoss, M. & Penn, D. J.** 2009. Females prefer the scent of outbred males: good-genes-as-heterozygosity? *BMC Evolutionary Biology*, **9**, 104, doi:10.1186/1471-2148-9-104.

**Johansson, B. G. & Jones, T. M.** 2007. The role of chemical communication in mate choice. *Biological Reviews*, **82**, 265–289, doi:10.1111/j.1469-185X.2007.00009.x.

**Johnston, R. E.** 2008. Individual odors and social communication: individual recognition, kin recognition, and scent over-marking. *Advances in the Study of Behavior*, **38**, 439–505, doi:10.1016/S0065-3454(08)00009-0.

**Jolly, A.** 1966. *Lemur Behavior: a Madagascar Field Study*. Chicago: University of Chicago Press.

**Lenochova, P., Roberts, S. C. & Havlicek, J.** 2008. Methods of human odor sampling: the effects of freezing. *Chemical Senses*, **34**, 127–138.

**Lynch, M. & Ritland, K.** 1999. Estimation of pairwise relatedness with molecular markers. *Genetics*, **152**, 1753–1766.

**Martin, P. & Bateson, P.** 1993. *Measuring Behaviour: an Introductory Guide*. Cambridge: Cambridge University Press.

**Mateo, J. M.** 2003. Kin recognition in ground squirrels and other rodents. *Journal of Mammalogy*, **84**, 1163–1181.

**Mathieu, E., Autem, M., Roux, M. & Bonhomme, F.** 1990. Épreuves de la validation dans l'analyse de structures génétiques multivariées: comment tester l'équilibre panmictique? *Revue de Statistique Appliquée*, **38**, 47–66.

**Mays, H. L. & Hill, G. E.** 2004. Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, **19**, 554–559, doi:10.1016/j.tree.2004.07.018.

**Meagher, S., Penn, D. J. & Potts, W. K.** 2000. Male–male competition magnifies inbreeding depression in wild house mice. *Proceedings of the National Academy of Sciences, U.S.A.*, **97**, 3324–3329.

**Milinski, M., Griffiths, S., Wegner, K. M., Reusch, T. B. H., Haas-Assenbaum, A. & Boehm, T.** 2005. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy of Sciences, U.S.A.*, **102**, 4414–4418, doi:10.1073/pnas.0408264102.

**Mitton, J. B., Schuster, W. S. F., Cothran, E. G. & De Fries, J. C.** 1993. Correlation between the individual heterozygosity of parents and their offspring. *Heredity*, **71**, 59–63.

**Neff, B. D. & Pitcher, T. E.** 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19–38, doi:10.1111/j.1365-294X.2004.02395.x.

**Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B. & Wittsell, H.** 2003. Major histocompatibility complex and mate choice in sand lizards. *Proceedings of the Royal Society B, Supplement*, **270**, S254–S256.

**Parga, J. A.** 2006. Male mate choice in *Lemur catta*. *International Journal of Primatology*, **27**, 107–131, doi:10.1007/s10764-005-9006-z.

**Parrott, M. L., Ward, S. J. & Temple-Smith, P. D.** 2007. Olfactory cues, genetic relatedness and female mate choice in the agile antechinus (*Antechinus agilis*). *Behavioral Ecology and Sociobiology*, **61**, 1075–1079, doi:10.1007/s00265-006-0340-8.

**Partridge, L.** 1983. Non-random mating and offspring fitness. In: *Mate Choice* (Ed. by P. Bateson), pp. 227–255. Cambridge: Cambridge University Press.

**Penn, D. J.** 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*, **108**, 1–21.

**Penn, D. J. & Potts, W. K.** 1999. The evolution of mating preferences and major histocompatibility genes. *American Naturalist*, **153**, 145–164.

**Pereira, M. E. & Weiss, M. L.** 1991. Female mate choice, male migration, and the threat of infanticide in ringtailed lemurs. *Behavioral Ecology and Sociobiology*, **28**, 141–152.

**Potts, W. K., Manning, C. J. & Wakeland, E. K.** 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature*, **352**, 619–621.

**Pusey, A. & Wolf, M.** 1996. Inbreeding avoidance in animals. *Trends in Ecology & Evolution*, **11**, 201–206.

**Queller, D. & Goodnight, K.** 1989. Estimating relatedness using genetic markers. *Evolution*, **11**, 201–206.

**Roberts, S. C. & Gosling, L. M.** 2003. Genetic similarity and quality interact with mate choice decisions by female mice. *Nature Genetics*, **35**, 103–106, doi:10.1038/ng1231.

**Schwensow, N., Fietz, J., Dausmann, K. & Sommer, S.** 2008. MHC-associated mating strategies and the importance of overall genetic diversity in an obligate pair-living primate. *Evolutionary Ecology*, **22**, 617–636, doi:10.1007/s10682-007-9186-4.

**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, doi:10.1016/j.anbehav.2006.08.006.

**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**, 493–504, doi:10.1093/chemse/bjm018.

**Silk, J. B., Altmann, J. & Alberts, S. C.** 2006a. Social relationships among adult female baboons (*Papio cynocephalus*) I. Variation in the strength of social bonds. *Behavioral Ecology and Sociobiology*, **61**, 183–195, doi:10.1007/s00265-006-0249-2.

**Silk, J. B., Alberts, S. C. & Altmann, J.** 2006b. Social relationships among adult female baboons (*Papio cynocephalus*) II. Variation in the quality and stability of social bonds. *Behavioral Ecology and Sociobiology*, **61**, 197–204, doi:10.1007/s00265-006-0250-9.

**Sokal, R. R. & Rohlf, F. J.** 1995. *Biometry*. New York: W.H. Freeman.

**Teriokhin, A. T., de Meeûs, T. & Guégan, J.-F.** 2007. On the power of some binomial modifications of the Bonferroni multiple test. *Journal of General Biology*, **68**, 332–340.

**Thom, M. D., Stockley, P., Jury, F., Ollier, W. E. R., Beynon, R. J. & Hurst, J. L.** 2008. The direct assessment of genetic heterozygosity through scent in the mouse. *Current Biology*, **18**, 619–623, doi:10.1016/j.cub.2008.03.056.

**Tiira, K., Laurila, A., Peuhkuri, N., Piironen, J., Ranta, E. & Primmer, C. R.** 2003. Aggressiveness is associated with genetic diversity in landlocked salmon (*Salmo salar*). *Molecular Ecology*, **12**, 2399–2407, doi:10.1046/j.1365-294X.2003.01925.x.

**Tiira, K., Laurila, A., Enberg, K., Piironen, J., Aikio, S., Ranta, E. & Primmer, C. R.** 2006. Do dominants have higher heterozygosity? Social status and genetic variation in brown trout, *Salmo trutta*. *Behavioral Ecology and Sociobiology*, **59**, 657–665, doi:10.1007/s00265-005-0094-8.

**Todrank, J. & Heth, G.** 2003. Odor–genes covariance and genetic relatedness assessments: rethinking odor-based "recognition" mechanisms in rodents. *Advances in the Study of Behavior*, **32**, 77–130.

**Tregenza, T. & Wedell, N.** 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, **9**, 1013–1027.

**Trivers, R.** 1985. *Social Evolution*. Menlo Park, California: Benjamin–Cummings.

**Van de Casteele, T., Galbusera, P. & Matthysen, E.** 2001. A comparison of microsatellite-based pairwise relatedness estimators. *Molecular Ecology*, **10**, 1539–1549.

**Wedekind, C. & Füri, S.** 1997. Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proceedings of the Royal Society B*, **264**, 1471–1479.

**Wedekind, C., Seebeck, T., Bettens, F. & Paepke, A. J.** 1995. MHC-dependent mate preferences in humans. *Proceedings of the Royal Society B*, **260**, 245–249.

**West, S. A., Pen, I. & Griffin, A. S.** 2002. Conflict and cooperation: cooperation and competition between relatives. *Science*, **296**, 72–75.

**Widdig, A.** 2007. Paternal kin discrimination: the evidence and likely mechanisms. *Biological Reviews*, **82**, 319–334, doi:10.1111/j.1469-185X.2007.00011.x.

**Widdig, A., Nürnberg, P., Krawczak, M., Streich, W. J. & Bercovitch, F. B.** 2001. Paternal relatedness and age proximity regulate social relationships among adult female rhesus macaques. *Proceedings of the National Academy of Sciences, U.S.A.*, **98**, 13769–13773.

**Willse, A., Kwak, J., Yamazaki, K., Preti, G., Wahl, J. H. & Beauchamp, G. K.** 2006. Individual odortypes: interaction of MHC and background genes. *Immunogenetics*, **58**, 967–982, doi:10.1007/s00251-006-0162-x.