

SHORT COMMUNICATION

Honest olfactory ornamentation in a female-dominant primateM. BOULET*†¹, J. C. CRAWFORD*¹, M. J. E. CHARPENTIER*‡§ & C. M. DREA*‡

*Department of Evolutionary Anthropology, Duke University, Durham, NC, USA

†Department of Biology, Bishop's University, Sherbrooke, Québec, Canada

‡Department of Biology, Duke University, Durham, NC, USA

§CEFE-CNRS, Montpellier, France

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Abstract

Sexual selection theory predicts that potential mates or competitors signal their quality to conspecifics. Whereas evidence of honest visual or vocal signals in males abounds, evidence of honest signalling via scent or by females is scarce. We previously showed that scent marks in male lemurs seasonally encode information about individual heterozygosity – a reliable predictor of immunocompetence and survivorship. As female lemurs dominate males, compete over resources, and produce sexually differentiated scent marks that likely evolved via direct selection, here we tested whether females also advertise genetic quality via olfactory cues. During the breeding season specifically, individual heterozygosity correlated negatively with the diversity of fatty acids (FAs) expressed in labial secretions and positively with the diversity of heavy FA esters. As odour–gene relationships predictive of health and survivorship emerged during a period critical to mate choice and female competition, we posit that genital scent marks function as honest olfactory ornaments in females.

Introduction

Elaborate ornaments often play an important role in mate choice by broadcasting information about an individual's genetic quality to potential mates or competitors (Amundsen, 2000). To be considered an 'honest signal', the quality of an ornament must be condition dependent, such that only the fittest individuals can bear the cost of producing high-quality signals (Andersson, 1994). Research on sexual selection is typically focused on female mate choice and male advertisement of competitive quality via elaborate ornaments; it too often neglects the potential function of female ornamentation. Nevertheless, there is theoretical (e.g. Chenoweth *et al.*, 2006) and empirical (Amundsen, 2000) evidence to suggest that female ornamentation may evolve via male

mate choice or female competition. Most indicators of female quality examined thus far, however, are visual (Amundsen, 2000; Kraaijeveld *et al.*, 2007; Huchard *et al.*, 2009) and typically correlate with other phenotypic proxies of quality (Amundsen & Forsgren, 2001; Siefferman & Hill, 2005; Weiss, 2006; Doutrelant *et al.*, 2008; Hanssen *et al.*, 2008). Evidence of a more direct relationship between ornament quality and genetic quality remains scarce (Amundsen, 2000).

Like visual cues, scent secretions may provide crucial information about prospective mates (Johansson & Jones, 2007) and function as reliable sexual ornaments (e.g. Rantala *et al.*, 2002). In previous analyses of the olfactory signals of a promiscuous primate, the ring-tailed lemur (*Lemur catta*), we showed that scrotal secretions encode information about male genetic diversity (i.e. genome-wide heterozygosity) during the competitive breeding season (Charpentier *et al.*, 2008a). As heterozygosity accurately predicts health and survivorship in male and female lemurs (Charpentier *et al.*, 2008b), we identified scrotal secretions as honest signals of male

Correspondence: Christine M. Drea, Department of Evolutionary Anthropology, Duke University, 129 Biological Sciences Bldg., Science Drive, Box 90383, Durham, NC 27708-0383, USA.

Tel.: +1 919 660 7367; fax: +1 919 660 7348; e-mail: cdrea@duke.edu

¹These authors contributed equally to this work.

genetic quality. These findings accord well with behavioural evidence in other species that genetically diverse individuals tend to be competitively superior (Meagher *et al.*, 2000; Tiira *et al.*, 2003).

Female lemurs are unusual among mammals in that they show some degree of sex reversal, including behavioural, physiological and anatomical masculinization (Drea, 2007; Drea & Weil, 2008). Because females are dominant to males, compete extensively with conspecifics over resources and produce seasonally and sexually distinct labial secretions (Scordato *et al.*, 2007; Boulet *et al.*, 2009), we predicted that female olfactory cues might also encode reliable information about genetic quality during the breeding season. In addition to correlating heterozygosity against the complete semiochemical profiles of female lemurs (as we had carried out in males), we also tested for biased expression of specific compounds that have been implicated in the signalling of female fertility. By showing that the genital scent marks of female lemurs honestly advertise genetic diversity, and hence individual health and survivorship, we highlight the importance of olfactory signals in mediating sexual selection, not only in males, but also in females.

Methods

Subjects and ethics statement

The study involved 17 reproductively intact, adult, female ring-tailed lemurs, aged 2–23 years. The lemurs were captive born and housed under varying social and spatial conditions (Scordato *et al.*, 2007) at the Duke Lemur Center (DLC; Durham, NC, USA). 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 USDA regulations. All research protocols were approved by Duke University IACUC (protocols #A245-03-07 and #A232-06-07).

Odourant sample collection and estimates of semiochemical diversity

We collected labial odourant samples from awake, manually restrained animals, from November 2003 to November 2007. We processed the samples and examined the volatile chemicals using gas chromatography and mass spectrometry (as per Scordato *et al.*, 2007; Charpentier *et al.*, 2008a; Boulet *et al.*, 2009). We quantified the complexity or diversity of female odourant profiles using the Shannon index (for a discussion of other such diversity indexes applied to chemical data, see Charpentier *et al.*, 2008a and Boulet *et al.*, 2009). The Shannon index takes into account both the number and relative abundance of compounds (PC-ORD 5.20; McCune *et al.*, 2002) and is a representative measure of the semiochemical diversity of lemur genital secretions

(scrotal: Charpentier *et al.*, 2008a; labial: Boulet *et al.*, 2009). Incorporating a measure of diversity, such as the Shannon index, avoids potential statistical issues that would otherwise arise as a result of analysing numerous variables (e.g. 338 compounds) in only a few subjects (e.g. 17 lemurs). Notably, this index transforms multiple variables into a single, intuitive measure (McCune *et al.*, 2002).

Estimates of female genetic diversity

To estimate genetic diversity, using genome-wide heterozygosity (H_O), we genotyped our subjects at 14 microsatellite loci (as per Boulet *et al.*, 2009; for details on methods and loci, see Charpentier *et al.*, 2008b). Female H_O (mean \pm SEM: 0.56 ± 0.03) ranged from 0.36 (relatively 'inbred') to 0.79 (relatively 'outbred') on a scale of 0–1. In our study population, H_O proved to be a good estimator of genome-wide inbreeding or loss in genetic diversity (Charpentier *et al.*, 2008b).

Statistical analyses

To test for odour–gene relationships in females during the breeding season, we performed Pearson correlations (CORR procedure, SAS version 9, SAS Institute Inc., Cary, NC, USA) between genetic diversity (H_O) and four measures of chemical diversity (each quantified via the Shannon index). Following the approach we had established for male data (Charpentier *et al.*, 2008a), we derived our first *a priori* measure of female chemical diversity using all volatile compounds expressed in labial secretions ($n = 338$, representing a broad range in molecular weights). Because several species of female primates are known to signal fertility through the expression of fatty acids (FAs; e.g. Michael *et al.*, 1974; Doty *et al.*, 1975; Matsumoto-Oda *et al.*, 2003), specifically, we expanded our original analysis with a second *a priori* measure of chemical diversity, derived using only this class of semiochemicals ($n = 33$ FAs, molecular weight range: 200–284).

Based on results from the analysis correlating H_O against FAs and from investigating the chromatograms, we then conducted two additional *post hoc* analyses using chemical diversity estimates derived from fatty acid esters (FAEs) – compounds commonly synthesized from FAs (Cheng & Russell, 2004; Hargrove *et al.*, 2004). Based on the bimodal distribution of their relative abundances and their molecular weights, we split FAEs into the following two classes of compounds: low-weight fatty acid esters (LFAEs, $n = 65$, molecular weight range: 298–396) and high-weight fatty acid esters (HFAEs, $n = 41$, molecular weight range: 424–522). Because of multiple testing, we performed a binomial test (following Teriokhin *et al.*, 2007) to determine whether the number of significant relationships was greater than expected by chance. We observed significantly more effects than expected by chance (number of successes: 2; number of trials: 4;

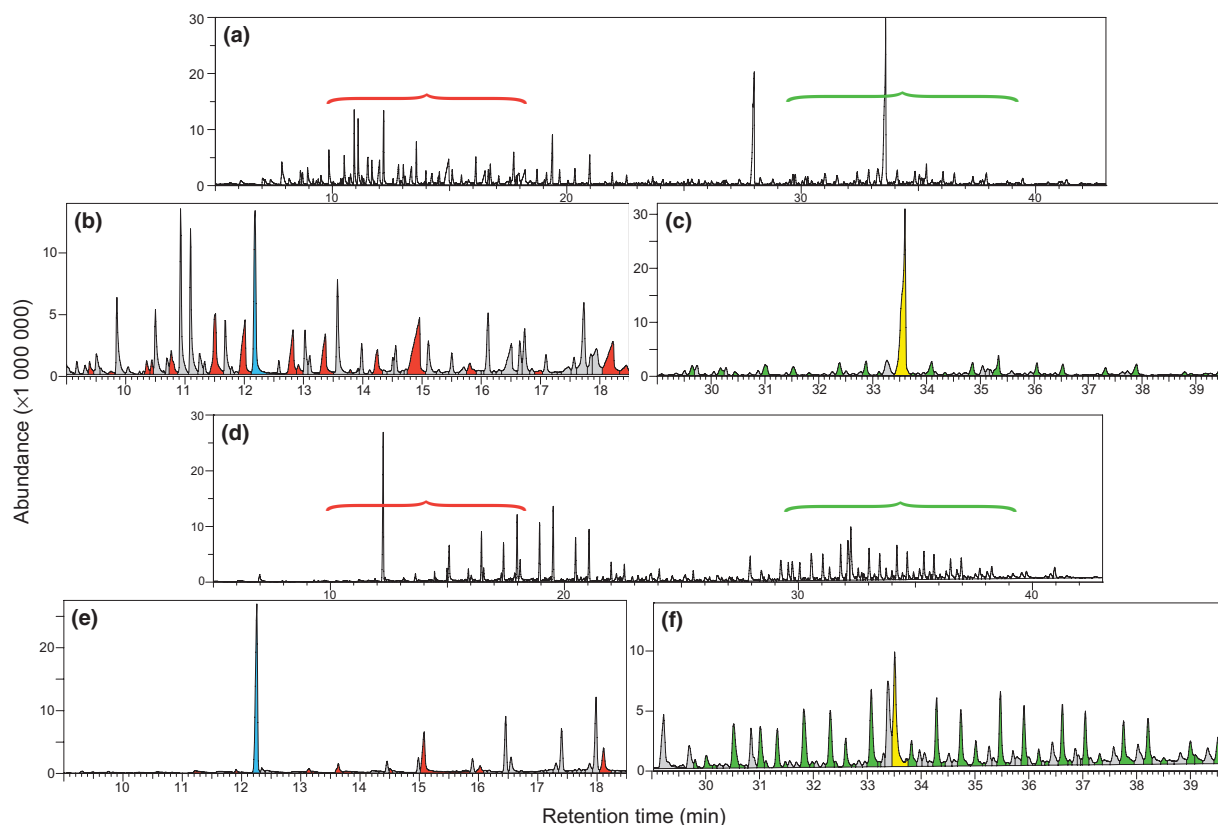


Fig. 1 Chromatograms of the labial secretions of two representative females differing by 0.43 in their genome-wide heterozygosity (H_O): (a, b, c) a relatively 'inbred' female with $H_O = 0.35$; (d, e, f) a relatively 'outbred' female with $H_O = 0.78$. Chromatograms (a) and (d) show the full complement of chemicals expressed in the two females, respectively (brackets show the retention ranges represented in greater detail below). Chromatograms (b) and (e) focus on the low molecular weight range of these females' respective chromatograms where fatty acids (FAs; red) occur, whereas (c) and (f) focus on the relatively high molecular weight range where FA esters (HFAEs; green) occur. The internal standard (hexachlorobenzene) is represented in blue; a naturally expressed compound (cholestanol), used as a reference, is represented in yellow.

threshold: 0.05; $P = 0.01$), suggesting that the relationships are biologically meaningful.

In a separate set of analyses, we repeated the above procedures using nonbreeding season semiochemical profiles, to verify that, as in males (Charpentier *et al.*, 2008a), female odour-gene relationships are absent outside of the breeding season.

Results and discussion

When we considered all 338 compounds expressed in female labial secretions during the breeding season (e.g. Fig. 1a,d), we found no covariation between chemical diversity and genetic diversity ($r = -0.10$; $P = 0.70$; $n = 17$ females); however, when we considered the diversity of FAs only, a strong, negative correlation emerged with H_O ($r = -0.61$, $P = 0.01$, Fig. 2a), such that a relatively inbred female expressed a high diversity of FAs (Fig. 1b), whereas a relatively outbred female expressed a low diversity of FAs (Fig. 1e). Thus, as in

males, an olfactory indicator of female genetic quality (i.e. one that is predictive of individual immunocompetence and survivorship) is available during the breeding season. Unlike in males, however, the indicator in females involves a specific class of compounds (rather than all compounds) and is negatively (rather than positively) correlated with heterozygosity.

In anthropoid primates, volatile FAs often vary with the female reproductive cycle, predicting fertility (Michael *et al.*, 1974; Doty *et al.*, 1975; Matsumoto-Oda *et al.*, 2003) and stimulating sexual interest in males (Michael & Keverne, 1968). Assuming the same relationships apply to strepsirrhine primates, one might have expected expression of more FAs in the fittest females (i.e. a positive correlation). With regard to signal quality, however, it may not be the case that 'more is better'. For instance, excessive female ornamentation may carry costs that are maladaptive (Chenoweth *et al.*, 2006), such that females may trade-off between allocating resources to reproduction versus to sexual signals

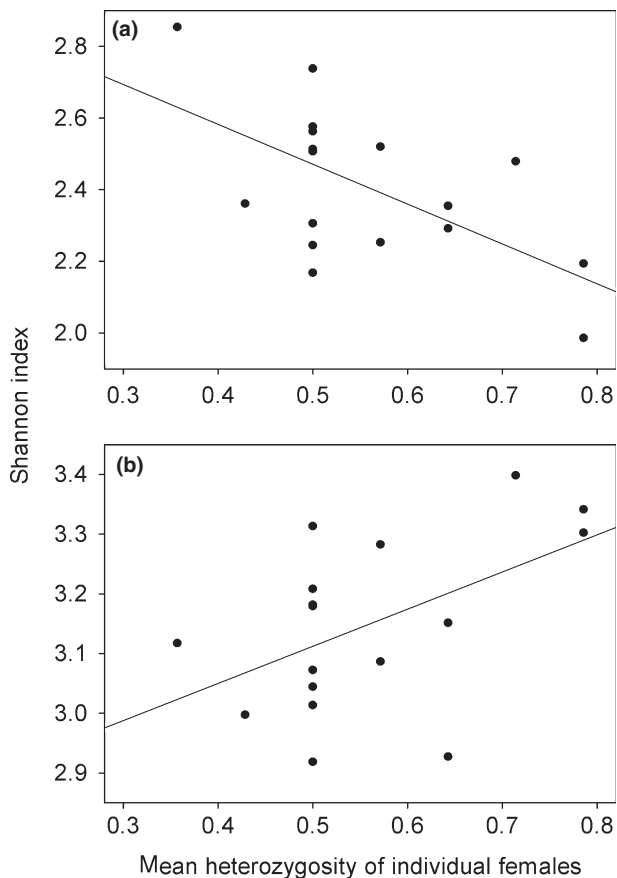


Fig. 2 Relationship between the genetic diversity of individual lemurs and the diversity of (a) fatty acids (FAs) or (b) high-weight FA esters (HFAEs) in their labial secretions.

(Chenoweth *et al.*, 2007). In addition, the presence of a greater diversity of compounds may increase the signal-to-noise ratio (e.g. Fig. 1b). Whereas the most heterozygous females expressed the lowest diversity of FAs, they also appeared to have concomitantly lost other highly volatile compounds that we were unable to identify (e.g. Fig. 1e). Thus, the dampening of low-molecular-weight 'noise' may have effectively produced a purer, albeit weaker, FA signal.

The relationship between genotype and chemical phenotype is undoubtedly complex and also may involve differential activation of certain metabolic pathways that influence the relative abundance of glandular semiochemicals (Albone, 1984; Smith & Thiboutot, 2008). In mammalian sebaceous glands, for instance, FAs can be esterified to synthesize FAEs (Cheng & Russell, 2004; Hargrove *et al.*, 2004). Interestingly, a *post hoc* investigation into the diversity of relatively high molecular weight FAEs revealed a seasonal relationship with genetic diversity. Specifically, the diversity of HFAEs during the breeding season correlated positively with H_O ($r = 0.51$, $P = 0.037$, Fig. 2b), such that relatively inbred females

expressed a low diversity of HFAEs (e.g. Fig. 1c), whereas relatively outbred females expressed a high diversity of HFAEs (e.g. Fig. 1f). In lemurs, the inverse relationship between the expression of FAs and HFAEs (Figs 1 and 2) likely reflects a biosynthetic trade-off, in which outbred females use FAs as building blocks to produce an olfactory signal that is enriched by HFAEs. As HFAEs are heavier than FAs, HFAEs would contribute to creating a less volatile (and potentially more expensive) signal. Accordingly, HFAEs may be the more stable and more salient cue of female quality.

Researchers have shown that olfactory cues correlate with potentially important phenotypic traits in both females and males of some vertebrate species. For instance, in turtles, the pheromonal blend produced by large females may indicate fecundity (Poschadel *et al.*, 2006). In lizards, the relative abundance of a chemical, cholesta-5,7-dien-3-ol, expressed in male femoral secretions correlates positively with T cell-mediated immune response (López & Martín, 2005); as this compound provides a reliable indicator of mate quality, female lizards prefer males that express this semiochemical in high abundance. To our knowledge, however, our study is the first to link characteristics of a female olfactory ornament to her genetic quality.

In explaining the evolution of honest female signals, two hypotheses have emerged. For instance, female ornaments may have evolved as a genetically correlated response to selection acting on male ornaments (Amundsen, 2000). This explanation is unsatisfactory for our study species, as the olfactory ornament of female ring-tailed lemurs is neither rudimentary nor nonfunctional. With regard to signal complexity, the genital signals of *L. catta* are sexually dimorphic, as the scrotal gland of males and the labial glandular fields of females derive from distinct tissues (Scordato & Drea, 2007; Drea & Weil, 2008) and express different compounds (Scordato *et al.*, 2007). In particular, females express a greater diversity of compounds in their genital secretions than do males (Boulet *et al.*, 2009), including 16 FAs and 11 HFAEs. With regard to signal functionality, female labial secretions generate significant interest in conspecifics and can signal reproductive condition, individuality (i.e. social status and familiarity; Scordato & Drea, 2007), relatedness and genetic diversity (Charpentier *et al.*, in press), suggesting that female genital odours serve multiple functions.

Alternatively, elaborate female ornaments may have arisen as a result of direct selection acting on females, owing to male mate choice or female contest competition (Amundsen, 2000). This explanation better fits the behavioural ecology of ring-tailed lemurs and accords well with behavioural observations of male mate choice in this species (Parga, 2006). Furthermore, we observed no odour-gene relationships during the nonbreeding season (all 338 compounds: $r = 0.01$; $P = 0.96$; FA: $r = 0.08$; $P = 0.75$; HFAE: $r = -0.07$; $P = 0.80$). Thus,

strong relationships between semiochemical diversity and genetic diversity appeared only in the breeding season – a highly stressful period (Starling *et al.*, 2010) – when such covariance would be critical to both male mate choice and female competition. Importantly, lemurs successfully differentiate the odours of inbred versus outbred females (Charpentier *et al.*, in press). We suggest, therefore, that as in males (Charpentier *et al.*, 2008a), the scent marks of female ring-tailed lemurs convey reliable and detectable information about the signallers' genetic quality and therefore function as honest olfactory ornamentation.

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