Smelling wrong: hormonal contraception in lemurs alters critical female odour cues

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Animals, including humans, use olfaction to assess potential social and sexual partners. Although hormones modulate olfactory cues, we know little about whether contraception affects semiochemical signals and, ultimately, mate choice. We examined the effects of a common contraceptive, medroxyprogesterone acetate (MPA), on the olfactory cues of female ring-tailed lemurs (*Lemur catta*), and the behavioural response these cues generated in male conspecifics. The genital odorants of contracepted females were dramatically altered, falling well outside the range of normal female variation: MPA decreased the richness and modified the relative abundances of volatile chemicals expressed in labial secretions. Comparisons between treatment groups revealed several indicator compounds that could reliably signal female reproductive status to conspecifics. MPA also changed a female's individual chemical 'signature', while minimizing her chemical distinctiveness relative to other contracepted females. Most remarkably, MPA degraded the chemical patterns that encode honest information about genetic constitution, including individual diversity (heterozygosity) and pairwise relatedness to conspecifics. Lastly, males preferentially investigated the odorants of intact over contracepted females, clearly distinguishing those with immediate reproductive potential. By altering the olfactory cues that signal fertility, individuality, genetic quality and relatedness, contraceptives may disrupt intraspecific interactions in primates, including those relevant to kin recognition and mate choice.

Keywords: olfactory communication; medroxyprogesterone acetate; mate choice; heterozygosity; kinship; primate

1. INTRODUCTION

A wide variety of animals, including humans, detect the fertility [1–4], quality [5–7] and compatibility [8–10] of potential mates through the assessment of conspecific olfactory cues. The natural hormonal modulation of odours provides a reliable mechanism for signalling reproductive potential, as scent signals vary with hormonal changes coincident with the breeding season [11,12] or, more specifically, with hormonal changes across the ovarian cycle [13]. Fluctuation in ovarian hormones is also linked to odour-mediated preferences for partner quality, as evidenced by cyclical shifts in female preference for scents associated with masculinity and symmetry [14]. As the endocrine system is implicated in both the expression and perception of odours, hormone manipulation, through the administration of contraceptives, could disrupt critical olfactory-mediated processes, including partner choice [15,16]. Hormone-based contraceptives are commonly used by humans (for contraceptive or hormonal therapy purposes, throughout the reproductive and post-menopausal lifespan) and are increasingly administered to captive and wild animals for conservation and management purposes [17]. Nevertheless, we know little about the extent to which hormonal contraception affects quantitative or qualitative

features of female olfactory cues. Here, using an integrative approach that combines hormonal, chemical, genetic and behavioural analyses, we document the profound impact of contraception on detectable female olfactory signals in a highly sociable, promiscuous primate, the ring-tailed lemur (*Lemur catta*).

In examining the effects of contraception on intersexual relationships, researchers typically ask whether the contraception of a female alters her behaviour or her attractiveness to males. For instance, hormonal contraception in women can reverse female preference for the scent of genetically dissimilar men ([15,18]; but see [19]) and diminish the male's ability to detect changes in the female's reproductive cycle [20,21]. Less attention, however, has been given to the chemical effects of contraception on the actual odours expressed by females. Consequently, it is unknown whether contraception only affects chemical signals of fertility or also affects olfactory signals encoding other socially relevant information, such as individuality, genetic quality and kinship.

Ring-tailed lemurs provide an ideal model for examining the effects of hormonal manipulation on olfactory signals, as they display a complex system of chemical communication that shows natural variation owing to hormonal modulation. In particular, lemur semiochemical profiles vary seasonally [22], paralleling changes in reproductive endocrine function [23]. Despite this variability, individual lemurs also maintain tractable semiochemical patterns across years, facilitating the long-term recognition of individuals [22,24]. These

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chemical data are well supported behaviourally, as lemurs discriminate genital secretions by the reproductive state, identity and sex of the secretion donor [25]. Lemurs additionally discriminate male and female odorants by both the neutral, genome-wide heterozygosity (H_O) of the signaller and the pairwise relatedness of the signaller–receiver dyad, suggesting that lemurs may use genetic information available in olfactory cues to choose the most appropriate social and sexual partners [10]. Because the chemical patterns by which lemurs encode genetic information have been well described [26–28], it is now possible to track hormonal influences on these patterns.

Here, we first characterize the modulation of sex steroid concentrations in adult, female lemurs following administration of a common hormonal contraceptive, medroxyprogesterone acetate (MPA). Using a within-subjects design, we then test for semiochemical differences between labial secretions expressed when females cycle normally (hereafter, ‘intact’ females) versus when females are hormonally contracepted (hereafter, ‘contracepted’ females). Beyond describing treatment effects on semiochemical expression and identifying chemical indicators of female reproductive status, we investigate the potential effects of MPA on the information typically encoded in lemur olfactory signals, including individual identity, genetic diversity and kinship. Lastly, we conduct behavioural bioassays, using an established signaller–receiver paradigm, to evaluate the males’ olfactory responsiveness to hormonal effects in conspecific females.

2. MATERIAL AND METHODS

(a) Subjects

The main subjects were 25 adult ring-tailed lemurs (12 females, 13 males) from the Duke Lemur Center (DLC) in Durham, NC, USA. The hormonal treatment involved sexually mature females (ranging in age from 1.5 to 22 years, with maturity being advanced in captivity [23]), each serving as a subject under both the intact and contraception conditions (see below). The behavioural bioassays involved the adult males (ranging in age from 2.5 to 25.5 years), each serving as a ‘recipient’ of female scent signals (see below). The scent samples for these bioassays derived from 8 of the 12 main female subjects, selected based on the male recipient’s lack of familiarity with the female donor. As described in turn for the different analyses below, we used various sample sizes, including subsets of the main subjects (e.g. for endocrine profiling) or larger subject pools from previously published data (e.g. for semiochemical or genetic profiling).

We conducted our study during the species’s breeding season, which in the Northern Hemisphere spans from late October to early February [23]; however, for comparative purposes, some of our analyses also include chemical data derived from previous studies of the same animals conducted in both the breeding and non-breeding seasons [22,26–28]. All of the subjects were socially housed, as described elsewhere [25]. The procedures were in accordance with regulations of the United States Department of Agriculture, and the research protocols were approved by the Institutional Animal Care and Use Committee of Duke University (protocol nos A245-03-07 and A232-06-07).

(b) Female hormonal treatments

The hormonal treatments spanned three consecutive annual breeding seasons. Some of the females ($n = 7$) served first as intact subjects, then as contracepted subjects; the others ($n = 5$) served first as contracepted subjects, then as intact subjects. Three of the females in the latter group experienced a second contraception cycle after their control cycle. Contracepted females received intramuscular injections of MPA (Depo-Provera, Pfizer, 150 mg ml⁻¹; 5 mg kg⁻¹) every 30–40 days, from early September through to early February. To avoid the significant variation in female hormone profiles introduced by pregnancy [29], most females in the intact condition were denied physical access to adult males during the breeding season; otherwise, for females that were allowed access to males, but conceived late in the breeding season, we used only samples collected in the first, non-conceptive breeding cycle (see below). Groups containing intact females and their young offspring were nonetheless housed adjacent to at least one adult male member of their normal social unit, separated only by chain-link fencing (see electronic supplementary material, figure S1). Thus, all females were regularly exposed to male visual, auditory and olfactory cues. Under these conditions, we see no effect of housing on chemical profiles (see the electronic supplementary material).

(c) Blood sample collection and endocrine assays

During the 2006–2007 breeding season, we obtained and processed blood samples (3 cm³), as described elsewhere [23], from a subset of female subjects representing each treatment group (five contracepted; four intact). Blood draws occurred 17 days after the MPA injections and were time-matched for the intact females. Serum 17 α -hydroxyprogesterone (OHP), Δ^4 androstenedione (androst-4-ene-3,17,dione; A_4), testosterone (T) and 17 β -oestradiol (E_2) were assayed at the Yerkes National Primate Research Center’s Endocrine Core Laboratory (Emory University, Atlanta, GA, USA), using commercially available radioimmunoassay kits (see the electronic supplementary material).

(d) Odorant sample collection and chemical assays

We collected and stored female odorant samples as described previously [25]. To avoid potential seasonal variation in reproductive cycle and odorant profiles, we obtained samples from all females within a narrow span of calendar days (e.g. 30 October–3 November) across years of the study. As females of this species are strictly seasonal and are roughly synchronous in their oestrous cycles [23], our breeding season samples were obtained from females that were in comparable stages of their reproductive cycles.

We used some of the samples for behavioural bioassays (see below); others we analysed using gas chromatography–mass spectrometry (GCMS), following previously published procedures ([22,26]; see the electronic supplementary material). We detected the volatile compounds using an automatic peak detector (SOLUTION WORKSTATION software, Shimadzu Scientific Instruments) and verified peaks individually by consulting both our own database and the National Institute of Standards and Technology library. As before [26–28], we excluded from our statistical analyses any peaks that comprised less than 0.05 per cent of the overall chromatogram area. For the three females that experienced two contraception cycles, we averaged the relative abundances obtained from their duplicate samples.

(e) Genetic analyses

Our genetic variables were extracted from a multigenerational dataset involving 81 individuals genotyped at 11–14 microsatellite loci [26,27,30]. As previously described, we had estimated genetic diversity using individual genome-wide heterozygosity (H_O) and had estimated kinship using female–female (FF) and female–male (FM) pairwise genetic distances (identity index [31]). For the females under study here, H_O varied from 0.43 to 0.79 on a scale of 0–1.

(f) Behavioural bioassays

We performed 34 behavioural bioassays for which we temporally isolated each male subject in his respective habitual enclosure. Using three fresh wooden dowels, aligned sequentially (as detailed in [25]), we presented the males with two odorant samples from the same female—one collected under intact and one under contraception conditions. Female odorants were placed on the two outer dowels (in random order), separated by a control cotton-scented dowel (see electronic supplementary material, video).

Although the collection dates of matched samples varied by one to three breeding seasons, we paired the females' samples such that they represented a comparable two-week period of the year. As lemurs maintain consistent individual 'signatures' across years [25], interannual variation in semiochemical profiles is minor. Female odorant 'donors' were unfamiliar to their male 'recipients', having never lived in the same social group. Females served as donors in 1 to 11 trials; males served as recipients in 1 to 5 trials. We videotaped the 10 min trials, and a rater blind to the treatments scored male behaviour, as described previously [10,25]. We estimated intraobserver reliability by calculating an index of concordance [32] for each behavioural measure. These indexes were as follows: sniff substrate = 91 per cent, sniff mark = 88 per cent, lick substrate = 100 per cent, lick mark = 100 per cent and proximity (exclusive of other behaviour) = 92 per cent.

(g) Statistical analyses**(i) Hormone analyses**

The endocrine data were normally distributed (Shapiro–Wilks test; $p > 0.05$; JMP 8.0, SAS Institute). As we collected the blood samples from different individuals, we used two-sample t -tests (JMP 8.0) to compare the steroid concentrations of intact and contracepted females. We predicted a decrease in OHP with MPA treatment (owing to a negative feedback effect), so our analysis of OHP was one-tailed; all other hormone analyses were two-tailed.

(ii) Semiochemical analyses

We first examined broad effects of contraception by comparing semiochemical profiles (i.e. the relative abundance of all compounds expressed) from the same females across three conditions: (i) contracepted during the breeding season ($n = 12$), (ii) intact during the breeding season ($n = 12$) and (iii) intact during the non-breeding season ($n = 11$; using data available from prior studies, as described in §2a). We compared these three conditions using principal component analysis (PCA; extracting components with eigenvalues greater than 1 that accounted for greater than 1 per cent of the variance; JMP 8.0), linear discriminant analysis, LDA (JMP 8.0) and Wilks's λ tests (as in [22]).

We next focused on differences in the chemical constitution of labial secretions from contracepted and intact females during the breeding season only. Specifically, we

compared chemical diversity or 'richness' [26] and relative abundance across treatments, using either the full complement of compounds or only certain classes of compounds [22,28]. These classes included alcohols, fatty acids, relatively low-molecular-weight fatty acid esters (LFAEs), and relatively high-molecular-weight fatty acid esters (HFAEs). We used matched-pairs t -tests for normally distributed data and Wilcoxon signed-rank tests for non-normally distributed data (JMP 8.0). Because of multiple testing, we performed binomial tests (following [33]; threshold: 0.05) to ensure that significant results were biologically meaningful.

To test whether any of the compounds expressed by females could be used as predictors of hormonal treatment, we used indicator species analysis (ISA; PC-ORD 5.19 [34,35]; see electronic supplementary material). As these analyses involved 390 comparisons, we used QVALUE [36] to estimate the minimum false discovery rate (q -value) of each ISA p -value.

Lastly, we tested for treatment effects on olfactory cues of individuality. To see whether contraception disrupted individual scent signatures, we used PCA, LDA and Wilks's λ tests, as in [22], to first confirm individual scent signatures in those females ($n = 3$) for which we had five to seven 'intact' samples (collected at different times of the year and across multiple years; data derived from this and prior studies, as described in §2a). We then repeated the analysis following inclusion of each female's respective sample collected during contraception. To see whether individual signatures might converge as a result of contraception, we used PC-ORD 5.19 [35] to compute chemical distances (relative Euclidean) for all possible dyads within each treatment group (for all 12 subjects; $n = 66$). For each subject in each treatment condition, we calculated her average chemical distance to all other subjects, and then compared these average chemical distances across treatments using a matched-pairs t -test.

(iii) Integrative analyses of odour–gene relationships

We previously documented a significant, positive correlation in female lemurs between the diversity of HFAEs in their genital secretions and genome-wide heterozygosity [28]. Using Pearson correlations (CORR procedure, SAS v. 9) on our normally distributed data (Shapiro–Wilks test; $p > 0.05$), we repeated this analysis to test for treatment effects on patterns of odour–heterozygosity covariance.

Next, we assessed treatment effects on odour–gene covariance, to see whether contraception degraded the olfactory information about a female's pairwise relatedness to female and male conspecifics. Using PERM [37], as in [27], we tested for differences between mean chemical distances (relative Euclidean) across five classes of pairwise genetic distances between FF dyads and FM dyads (see also the electronic supplementary material). The FF analyses included three comparisons: (i) 66 intact–intact dyads, F_iF_i (to confirm that prior findings maintained despite a smaller sample size); (ii) 66 intact–contracepted dyads, F_iF_c ; and (iii) 66 contracepted–contracepted dyads, F_cF_c . The FM analyses included two comparisons: (i) 228 intact female–male dyads, F_iM (again, confirming prior findings); and (ii) 228 contracepted female–male dyads, F_cM . Each randomization test included 10 iterations of 1000 permutation events. Across all iterations, p -values were averaged. We performed binomial tests (threshold: 0.05) to ensure that results were biologically meaningful.

(iii) *Behavioural analyses*

To determine whether male lemurs discriminated between odorants based on the donor female's treatment condition, we compared the duration with which male recipients directed behaviour towards each type of sample. We used logarithmic or reciprocal transformations to achieve normality for non-normally distributed data. We then used mixed models (mixed procedure, SAS 9.1) to assess differential behaviour allocations towards the two types of odorants. To correct for the non-independence of data points, we classified both the receiver and the donor as random effects. We used Glass's Δ as an estimator of effect size and a binomial test (threshold: 0.05) to ensure that significant results were biologically meaningful.

3. RESULTS

(a) *Effects of MPA on female endocrine profiles*

As anticipated, contraception had relatively modest effects on steroid concentrations (figure 1). Compared with their intact condition, contracepted females showed significantly reduced serum OHP concentrations ($t_7 = 2.36$, $p < 0.05$). Although contracepted females also tended to show reduced A_4 concentrations ($t_6 = 1.30$, $p = 0.12$, n.s.) and elevated E_2 concentrations ($t_7 = 1.05$, $p = 0.16$, n.s.), neither pattern achieved statistical significance. Lastly, we found no difference in T concentrations between treatment groups ($t_7 = 0.65$, $p = 0.54$, n.s.).

(b) *Effects of MPA on female odour profiles*

By contrast with the effects on hormone profiles, MPA dramatically altered the olfactory profiles of female lemurs (figure 2). A comparison of contracepted females against intact females in both the breeding and non-breeding seasons revealed a significant change in the principal semiochemical components (Wilks's $\lambda = 0.004$, $p < 0.01$; figure 3a). Contraception thus altered the relative abundances of expressed compounds in a manner that was not merely characteristic of the non-breeding season (figure 3a).

A more detailed comparison of contracepted and intact females during the breeding season revealed that, of all the detectable compounds expressed ($n = 390$), 35 (9%) and 17 (4.4%) were unique to the contracepted and intact conditions, respectively. Thus, a conspecific could rely on the presence or absence of minimally 13.3 per cent of the compounds in labial secretions to evaluate a female's fertility. Moreover, contraception decreased the total number of compounds expressed by females (Wilcoxon $t_{11} = 22$, $p < 0.05$; figure 4a) and resulted in the differential expression of several classes of compounds (binomial test: $p < 0.001$; figure 4b–f). For instance, contracepted females expressed fewer alcohols (matched pairs $t_{11} = 3.25$, $p < 0.01$), as well as reduced relative abundances of alcohols (Wilcoxon $t_{11} = 28$, $p < 0.05$), fatty acids (Wilcoxon $t_{11} = 1.80$, $p < 0.05$) and LFAEs (matched pairs $t_{11} = 2.42$, $p < 0.05$). Females tended to express a greater abundance of HFAEs while contracepted, but not reliably so ($t_{11} = 2.09$, $p = 0.06$, n.s.).

The effect of contraception on olfactory cues was so pervasive that even semiochemical compounds expressed by all females reliably predicted treatment condition via changes in their relative abundances. For instance, of all

Figure 1. Serum concentrations (mean $\pm$ s.e.m.) of various sex steroids (including hydroxyprogesterone, androstenedione, testosterone and oestradiol) in hormonally intact (black bar) and hormonally contracepted (white bar) female ring-tailed lemurs. Asterisk indicates a significant difference ($p < 0.05$) based on a one-tailed two-sample t -test.

the detectable compounds ($n = 390$), 31 (7.9%) were significant indicators of the intact condition and 25 (6.4%) were significant indicators of the contraception treatment (all p -values < 0.05 ; average q -value $\pm$ s.e.m.: 0.18 ± 0.04 ; see the electronic supplementary material). Of these 56 significant indicators, 49 were expressed by females regardless of treatment. Thus, a conspecific could rely on the relative abundance of an additional 12.6 per cent of the compounds present in labial secretions to evaluate a female's fertility.

(c) *Effects of MPA on individual scent signatures*

Remarkably, contraception also eliminated individual-specific olfactory information (figure 3b). For instance, the three females that had sufficient sample representation under the intact condition showed significant individual semiochemical signatures (Wilks's $\lambda = 0.038$, $p < 0.05$), consistent with previous findings in a larger study [22]. These signatures were lost, however, by adding to the analysis a single contraception sample per individual (Wilks's $\lambda = 0.245$, $p = 0.228$, n.s.). Moreover, the average ($\pm$ s.e.m.) chemical distances between females decreased significantly under contraception (intact: 0.841 ± 0.02 ; contracepted: 0.713 ± 0.03 ; matched pairs, $t_{11} = -5.16$, $p \leq 0.001$), indicating a convergence among the olfactory profiles of contracepted females.

(d) *Effects of MPA on odour signals of genetic quality*

Beyond the quantitative and qualitative effects of MPA on female odorants, contraception also eliminated the honest olfactory signalling of female genetic diversity. As in a prior study [28], we found a significant positive correlation between the diversity of HFAEs and H_O in intact females ($r = 0.59$, $p < 0.05$); however, the correlation was lost when the same females were contracepted ($r = 0.27$, $p = 0.387$, n.s.; figure 5). Therefore, the otherwise reliable olfactory cues of genetic quality (i.e. diversity) disappeared under contraception.

(e) *Effects of MPA on signals of kinship*

Lastly, hormonal contraception diminished chemical signals of relatedness, both within and between the sexes. The chemical distance between females reliably differed across classes of pairwise genetic distance when

Figure 2. Chromatograms of the labial secretions of two representative ring-tailed lemurs while (a,c) hormonally intact and (b,d) hormonally contracepted (rows represent different individuals, columns represent different treatments). Letters identify the following compounds: a, tetradecanoic acid; b, internal standard (hexachlorobenzene); c, pentadecanoic acid; d, 1-hexadecanol; e, n-hexadecanoic acid; f, octadecanoic acid; g, squalene; h, hexadecanoic acid, tetradecyl ester; i, hexadecanoic acid, hexadecyl ester; j, unidentified fatty acid ester. Based on their relative abundances, compounds c and d are indicators of the intact treatment, whereas compounds i and j are indicators of the contracepted treatment. Compound j was also expressed solely by contracepted females.

comparing $F_i F_i$ (randomization tests: $p \leq 0.001$ for 7/10 class comparisons; binomial test: $p < 0.001$) or $F_i F_c$ (randomization tests: $p \leq 0.001$ for 7/10 comparisons; binomial test: $p < 0.001$) dyads, but not when comparing $F_c F_c$ dyads (randomization tests: $p > 0.05$ for 10/10 comparisons, n.s.; binomial test: $p = 0.599$, n.s.). Similarly, the chemical distances between females and males differed across classes of genetic distance in $F_i M$ dyads (randomization tests: $p \leq 0.001$ for 9/10 comparisons; binomial test: $p < 0.001$), but not in $F_c M$ dyads (randomization tests: $p > 0.05$ for 8/10 comparisons, n.s.; binomial test: $p = 0.075$, n.s.). Contraception therefore attenuated chemical signals of kinship to female and male conspecifics.

(f) Male detection of MPA effects

Male ring-tailed lemurs discriminated hormonal treatment in female scent, showing clear biases for the odour of intact over contracepted females (figure 6). Relative to the odorants of intact females, males spent less time investigating (sniffing + licking: Glass's $\Delta = -0.41$; $F_{1,47} = 5.72$, $p < 0.05$) and less time near (Glass's $\Delta = -0.62$; $F_{1,47} = 12.37$, $p < 0.01$) the odorants of contracepted females (binomial test: $p < 0.05$).

4. DISCUSSION

Despite the relatively modest changes that modern hormonal contraceptives have on female reproductive hormones, these same hormonal contraceptives can nonetheless drastically alter female olfactory profiles. We found that semiochemical expression in female ring-tailed lemurs was both depressed and modified as a function of MPA contraception, and that various compounds emerged as reliable indicators of female reproductive potential. The contraception of female lemurs altered more than chemical correlates of fertility, however: remarkably, contraception ablated or depressed a female's chemical encoding of (i) identity or individuality, (ii) genetic quality, and (iii) pairwise relatedness to both female and male conspecifics. As males were able to detect these hormonally induced changes in female scent, we suggest that contraception could negatively influence a far broader range of odour-mediated social behaviour than expected, ultimately undermining kin recognition and mate choice. Given the common usage of hormonal contraceptives by humans, it would be intriguing to know whether comparable data might be obtained in women.

With regard to fertility, contraception may diminish the otherwise salient, hormonally mediated cues that signal an individual's reproductive potential. In women,

Figure 3. Linear discriminant analyses (LDA) of the principal components derived from the relative abundances of semiochemicals expressed in the genital secretions of female ring-tailed lemurs. (a) LDA plot of the semiochemical components expressed by contracepted females (white circle) versus intact females during the breeding (black circle) and non-breeding (black triangle) seasons. (b) LDA plot of the semiochemical components expressed by three females (each represented by diamonds, triangles and rectangles, respectively) while intact across multiple years (black symbol) versus when contracepted (white symbol). To illustrate the differences in contracepted and intact samples, data points in (b) are grouped by both individual and treatment.

for instance, features such as facial appearance [38], vocal pitch [39], attractiveness of axillary sweat [1,20,40] and fatty acid content in vaginal secretions [41] change noticeably across phases of the menstrual cycle, such that they may function as indicators of fecundity. Often, these qualitative changes parallel quantitative changes in oestrogen and progesterone concentrations [13,42], and, interestingly, are either diminished [20] or absent [43] in contracepted women. We found a similar correlation between hormonal status and olfactory indicators of fertility in lemurs, with the manipulation introduced by contraception implying a causal relationship. Relative to intact females, for instance, the semiochemical profiles of contracepted females were shifted towards the expression of heavier, less volatile compounds (such as HFAEs), at the expense of lighter, more volatile compounds (such as fatty acids and LFAEs). Interestingly, volatile fatty acids have been suggested to signal fertility in humans and other anthropoid primates [41,44], consistent with the present findings. Moreover, approximately 26 per cent of the compounds expressed in lemur labial secretions (either via their presence/absence or via their relative abundance) were reliable predictors of female reproductive state. Whether male lemurs were tuned to specific chemicals or overall profiles is unclear, but they clearly distinguished between the odours of contracepted and intact females.

Figure 4. Quantitative measures (mean $\pm$ s.e.m.) of chemicals expressed in the genital secretions of female ring-tailed lemurs while hormonally intact (black bar) and hormonally contracepted (white bar). Comparisons are provided for the total number or 'richness' of (a) all compounds and (b) alcohols detected across treatments. Also shown is variation in the relative abundance of specific classes of compounds, comprising (c) alcohols, (d) fatty acids, (e) LFAEs, and (f) HFAEs, across treatments. Asterisks indicate significant differences at $*p < 0.05$ or $**p < 0.01$ based on two-tailed Wilcoxon (a,d) and matched-pairs (b,c,e,f) tests.

The correlates of fertility that change under contraception are often likened to changes that might occur during pregnancy, as researchers often suggest that contraception hormonally mimics pregnancy (e.g. [16]). Nonetheless, hormonal contraception typically maintains relatively low progesterone and oestrogen concentrations, comparable with the early follicular or late luteal phases of the ovarian cycle, whereas pregnancy induces dramatic increases in these and other hormones across gestation [45]. The relatively low or even depressed steroid concentrations in contracepted lemurs, as found here, likewise bear little resemblance to the two- to fourfold increases in steroid concentrations observed in pregnant lemurs [29]. Moreover, based on preliminary comparisons, we see no convergence between the semiochemical profiles of contracepted and pregnant female lemurs. These observations suggest distinct hormonal and chemical differences between contraception and pregnancy.

Beyond affecting signals of fertility, contraception had dramatic effects on signals of identity. Individual scent signatures have been shown, either chemically or behaviourally, to characterize a wide range of social species (bats [46]; voles [47]; spotted hyaenas [48]; lemurs [22,24]; humans [49]), suggesting that communication of identity is critical for maintaining social relationships,

Figure 5. The relationship between genetic diversity (genome-wide heterozygosity) and diversity (Shannon index) of heavy fatty acid esters in (a) hormonally intact ($r = 0.59$, $p < 0.05$) and (b) hormonally contracepted ($r = 0.27$, $p = 0.387$) female ring-tailed lemurs, based on Pearson correlations.

Figure 6. Duration (mean $\pm$ s.e.m.) of the behavioural responses of male ring-tailed lemurs to the labial secretions of hormonally intact (black bar) and hormonally contracepted (white bar) females. The category of investigation combines sniffing and licking. Asterisks indicate significant differences at * $p < 0.05$ or ** $p < 0.01$ based on mixed models (see §2).

even between members of individualized societies. In lemurs, contraception altered otherwise stable patterns in female scent signatures (effectively changing their normal chemical identity) and decreased the chemical distance between females (effectively degrading the individual distinctiveness of their odours). As ring-tailed lemurs do not encode social status in their genital

secretions [22], they may use individual olfactory signatures to identify group members and navigate social hierarchies. By altering an individual's chemical uniqueness, hormonal contraceptives could have detrimental effects on social interactions, which may help explain the increase in aggression sometimes associated with MPA contraception in other primates [50,51].

Perhaps even more remarkably, contraception altered the accurate and honest olfactory communication of genetic information. Honest advertisement is key to selecting the most appropriate social and sexual partners [52–54], as mating with genetically incompatible individuals entails dire reproductive consequences, such as foetal loss (humans [55]; macaques [56]). Female choice of high-quality [6,7] or compatible [8] male partners is well established across vertebrate and invertebrate taxa, and there is likewise mounting evidence across various vertebrates of male preference for high-quality [57,58] or genetically compatible [59] females. Consistent with sexual selection operating in both sexes, we have found that both male and female lemurs encode within their volatile genital secretions critical genetic information, including quality [26,28] and kinship [26,27], which conspecifics of either sex can detect through olfactory investigation [10]. Therefore, both sexes may use olfactory-encoded estimates of genetic quality and pairwise relatedness to process trade-offs between selection for the best or most genetically diverse social or sexual partner, versus selection for the most compatible partner [10]. Here, we found that contraception may undermine the ability to process such trade-offs, as olfactory signals of genetic quality and kinship were lost or diminished under hormonal treatment.

As in lemurs, human olfactory cues communicate a variety of important information, including reproductive status [1,20,40], identity [49,60], genetic quality [5,14] or MHC compatibility (reviewed in [9]) and kinship [60,61]. Humans also engage in disassortative mating on the basis of genetic compatibility [55,62], potentially relying on signal concordance between visual and olfactory modalities when choosing a partner [63]. Future research should be aimed at determining whether the effects of contraception on human olfactory signals compare with those we have described for lemurs. If such were the case, the use of MPA-containing hormonal contraceptives by humans could disrupt the olfactory communication of fertility, individuality, genetic quality and kinship.

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