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MHC GENETIC VARIATION INFLUENCES BOTH OLFACTORY SIGNALS AND SCENT DISCRIMINATION IN RING-TAILED LEMURS

RUNNING TITLE: MHC signaling & discrimination in Lemur catta

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ABSTRACT (Limit 250 words)

Diversity at the Major Histocompatibility Complex (MHC) is critical to health and fitness, such that MHC genotype may predict an individual's quality or compatibility as a competitor, ally, or mate. Moreover, because MHC products can influence the components of bodily secretions, an individual's body odor may signal its MHC and influence partner identification or mate choice. To investigate MHC-based signaling and recipient sensitivity, we test for odor-gene covariance and behavioral discrimination of MHC diversity and pairwise dissimilarity, under the good genes and good fit paradigms, in a strepsirrhine primate, the ring-tailed lemur (*Lemur catta*). First, we coupled genotyping with gas chromatography-mass spectrometry to investigate if diversity of the MHC-DRB gene is signaled by the chemical diversity of lemur genital scent gland secretions. We also assessed if the chemical similarity between individuals correlated with their MHC similarity. Next, we assessed if lemurs discriminated this chemically encoded, genetic information in opposite-sex conspecifics. We found that both sexes signaled overall MHC diversity and pairwise MHC similarity via genital secretions, but in a sex- and season-dependent manner. Additionally, both sexes discriminated absolute and relative MHC-DRB diversity in the genital odors of opposite-sex conspecifics, supporting previous findings that lemur genital odors function as advertisement of genetic quality. In this species, genital odors provide honest information about an individual's absolute and relative MHC quality. Complementing evidence in humans and Old World monkeys, our results suggest that reliance on scent signals to communicate MHC quality may be important across the primate lineage.

INTRODUCTION

The Major Histocompatibility Complex (MHC) is an extremely polymorphic group of genes within the adaptive immune system of vertebrates that plays a critical role in disease resistance (Piertney & Oliver 2006). Because genetic diversity at the MHC is fundamentally linked to parasite resistance, survivorship, and reproductive success (Sommer 2005a, Piertney & Oliver 2006), an individual's MHC genotype is hypothesized to be an important predictor of its quality as a mate. If recognizable to others, animals could increase their reproductive success by selecting mates that possess particular MHC genotypes, such as diverse alleles or specific disease-resistant alleles (Penn & Potts 1999, Mays & Hill 2004). Although researchers have found evidence that MHC genotype influences mate choice or its proxies in many species (reviewed in Kamiya et al. 2014), the mechanism by which animals assess the MHC of conspecifics is still under investigation (reviewed in Ruff et al. 2012). Given that the protein products of the MHC can influence body odor, scientists have implicated an olfactory-based mechanism (reviewed in Ziegler et al. 2005, Boehm & Zufall 2006); however, researchers rarely combine chemical and behavioral approaches within the same study to test the purported mechanism of information transfer (Leinders-Zufall et al. 2004, Milinski et al. 2005). Here, using the ring-tailed lemur (*Lemur catta*) – a strepsirrhine primate for which there is strong evidence of condition-dependent olfactory signaling (Charpentier et al. 2008, Boulet et al. 2010, Crawford et al. 2011) – we test for olfactory-based MHC advertisement and recognition. Specifically, we combine MHC genotyping with chemical analyses of genital secretions and with behavioral tests of scent discrimination between opposite-sex conspecifics to ask 1) if lemurs advertise their genetic quality and similarity at the MHC via chemical cues and 2) if conspecifics can detect this olfactory information.

Because an offspring's health is influenced by the genotypes inherited from its parents, extreme polymorphism of the MHC is likely to be maintained both by health-mediated natural selection and by MHC-based sexual selection (Winternitz et al. 2013). Potential mates might be

chosen for their MHC diversity, for their possession of a particular disease-resistant allele or for their MHC dissimilarity relative to the chooser (Sommer 2005a, Milinski 2014). Two models have been developed to explain the types of genetic benefits accrued owing to the potential partner's genotype: In the 'good genes' model, individuals, regardless of their own genotype, choose 'high-quality' mates, with quality being defined by absolute or optimal genetic diversity or by possession of a particularly beneficial allele (Andersson 1994, Penn 2002, Neff & Pitcher 2005, Kempenaers 2007). This model is supported by research in several species of fish (Forsberg et al. 2007, Eizaguirre et al. 2009, Evans et al. 2012), and birds (Ekblom et al. 2004, Richardson et al. 2005).

In the 'good fit' model, individuals choose mates based on their relative genetic compatibility (Trivers 1972, Brown 1999, Neff & Pitcher 2005), so as to optimize parental genetic dissimilarity, minimize the risk of inbreeding or increase the genetic diversity of their offspring (Tregenza & Wedell 2000, Penn 2002). Support for this model (reviewed in Kamiya et al. 2014) derives from species of fish (Landry et al. 2001, Aeschlimann et al. 2003, Neff et al. 2008), reptiles (Olsson et al. 2003), birds (Bonneaud et al. 2006, Freeman-Gallant et al. 2003), and mammals (Radwan et al. 2008, Burger et al. 2015), including primates (Ober 1999, Schwensow et al. 2007 & 2008, Setchell et al. 2010, Kromer et al. 2016). The good genes and good fit models are not mutually exclusive, however, because a potential mate could be both maximally diverse and optimally dissimilar (Mays & Hill 2004). Individuals may also alter their preferences based on environmental conditions, current pathogen pressures or potential risk of inbreeding (Qvarnstrom 2001, Mays & Hill 2004).

Regardless of the model, for mate choice to be influenced by the MHC, individuals must both signal their respective MHC genotype and be able to evaluate the MHC information in the signals of conspecifics (Boehm & Zufall 2006, Hettyey et al. 2010). Previously, researchers have shown that condition-dependent signals of quality can be used by both sexes to assess potential mates (Penn & Potts 1998a & 1998b, Charpentier et al. 2010, Johansson & Jones

2007). Although evidence of correlation with MHC genotype has derived primarily from visual signals, such as antler size (Ditchkoff et al. 2001) or bright coloration (Setchell et al. 2009), chemical signals could prove more reliable for advertising MHC genotype (Blaustein 1981, Penn 2002, Mays & Hill 2004, Ziegler et al. 2005): Notably, because degraded MHC molecules are shed from the cell surface and found in body fluids (e.g., serum, saliva, sweat, urine, and glandular secretions), they may function directly as odorants (Singh et al. 1987, Milinski et al. 2005, Boehm & Zufall 2006). MHC molecules may also bind relevant volatile compounds (Leinders-Zufall et al. 2004, Willse et al. 2005, Aksenov et al. 2012, but see Kwak et al. 2010). Lastly, the MHC may influence the composition of the host's microbiota (Lanyon et al. 2007, Zomer et al. 2009, Archie & Theis 2011), including those dwelling within scent glands that contribute to volatile chemical production (Gorman et al. 1974, Theis et al. 2013, Leclaire et al. 2017a & 2017b). Among taxa that display MHC-associated mate choice, researchers have implicated the operation of an olfactory mechanism in fish (Reusch et al. 2001, Aeschlimann et al. 2003, Milinski et al. 2005), reptiles (Olsson et al. 2004), birds (Ekblom et al. 2004, Leclaire et al. 2014), and mammals (Yamazaki et al. 1976, Radwan et al. 2008), including humans (reviewed in Havlicek & Roberts 2009, Winternitz et al. 2016).

In terms of a model species for an odor-based test of MHC signaling (e.g., Knapp et al. 2006) and discrimination of mate quality, ring-tailed lemurs are ideal because of their elaborate system of olfactory communication (Jolly 1966). Animals of both sexes engage in genital scent marking, depositing chemically complex secretions that share ~170 volatile compounds (Scordato et al. 2007). The diversity and relative abundance of these chemicals contain information about the signaler's sex, breeding condition, individual identity, and genome-wide microsatellite diversity (or neutral heterozygosity), as well as its relatedness to other individuals (Scordato et al. 2007, Charpentier et al. 2008, Boulet et al. 2009 & 2010, Crawford et al. 2011). Moreover, this chemically encoded information is salient and distinguishable to conspecifics (Scordato & Drea 2007, Charpentier et al. 2010, Crawford et al. 2011). Thus, lemur genital

122 odors honestly advertise at least one measure of genetic quality and relatedness in both sexes.
123 To test if ring-tailed lemurs also advertise their MHC quality and dissimilarity via olfactory
124 signals, we genotyped animals at the most diverse MHC gene, DRB, analyzed the volatile
125 chemical composition of their genital secretions, and used behavioral testing to determine if
126 conspecifics can use genital scent to discriminate between MHC genotypes, according to either
127 the good genes or good fit models.
128
129 **METHODS**
130
131 *Subjects*
132 Our subjects (*N* = 62) derived from three captive populations of ring-tailed lemurs, located
133 at the Duke Lemur Center (DLC, *N* = 24 females and 24 males) in Durham, NC, the Indianapolis
134 Zoo (*N* = 8 females and 4 males) in Indianapolis, IN, and the Cincinnati Zoo (*N* = 2 females) in
135 Cincinnati, OH. All of the animals were healthy adults that were reproductively intact (i.e.,
136 neither gonadectomized nor hormonally contracepted) at the time of study. They were housed in
137 mixed-sex pairs or groups, with similar living conditions and provisioning routines across all
138 three institutions (for more details, see Scordato et al. 2007). Animal care met with institutional
139 guidelines and was in accordance with regulations of the United States Department of
140 Agriculture. The research protocols were approved by the Institutional Animal Care and Use
141 Committee of Duke University (protocol numbers A245-03-07 & A143-12-05) and by the
142 research directors of each zoo.
143 As described below, although all individuals were genotyped for MHC-DRB diversity, not
144 all individuals participated as secretion donors for chemical analyses or bioassay presentation,
145 nor did all individuals participate as bioassay recipients. To achieve appropriate sample sizes
146 while working with an endangered species often requires years of sample collection and
147 observation, which presents various logistical challenges (for more details, see Drea et al.

148 2013). Specific to our work here, secretion sampling is restricted because of several factors,
149 including but not limited to facility practices which limit captures in order to avoid undue stress
150 for the animals, and unavailability of an animal due to hormonal contraception (Crawford et al.
151 2011), pregnancy (Crawford & Drea 2015), mortality, or transfer between facilities. Participation
152 as a bioassay recipient may also be precluded because of participation in previous studies
153 leading to a lack of any 'unknown' donor odors to present. For this study, however, we have
154 overcome these challenges wherever possible through long-term study or the addition of
155 animals from other facilities for use as participants.
156
157 *MHC genotyping*
158 Using DNA extracted from whole blood or tissue, we genotyped the subjects at the MHC-
159 DRB loci using parallel tagged next-generation sequencing (Grogan et al. 2016). Briefly, we
160 amplified a 171-bp fragment, excluding primers, of the 270-bp second exon of the MHC-DRB
161 gene. This fragment is the most frequently genotyped MHC loci in non-model primate species,
162 especially in lemur species for which genomic data to design primers are scarce (e.g.,
163 Schwensow et al. 2007, Huchard et al. 2012, Sommer et al. 2013, Pechouskova et al. 2015,
164 Kaesler et al. 2017). Because this fragment excludes several variable amino acids within the
165 MHC-DRB gene, we are likely underestimating the amount of MHC-DRB variability present.
166 Nonetheless, because the genotyped fragment represents the most variable part of exon 2, we
167 can use this 171-bp fragment as a proxy of diversity across the 6 exons of MHC-DRB. We
168 sequenced pooled amplicons using parallel tagged sequencings on two platforms: Ion Torrent
169 PGM® 314v2 chips (Life Technologies, Grand Island, NY, USA) and 454 Titanium® 1/8th lanes
170 (Roche, Nutley, NJ, USA). True MHC-DRB alleles were distinguished from artefacts using a
171 published workflow (Grogan et al. 2016). Each ring-tailed lemur possessed a mean ± S.D. of
172 2.22 ± 0.92 MHC-DRB alleles (range = 1-4; see Supplemental Table 1, adapted from Grogan et
173 al. 2017). Next, because of the degeneracy of the genetic code and the similarity in the

174 physiochemical properties of some amino acids, we organized these MHC-DRB alleles (*N* = 20)
175 into MHC-DRB 'supertypes' (*N* = 13; Grogan et al. 2017). Supertypes are groups of MHC alleles
176 that have similar antigen binding properties despite having different nucleotide sequences
177 (Doytchinova & Flower 2005, Schwensow et al. 2007), and thus are likely to bind the same
178 subset of pathogen peptides. Supertypes are determined first by aligning the MHC-DRB
179 sequences with the human HLA-DRB sequence (Brown et al. 1993) to identify antigen binding
180 sites. Then, amino acid sites under positive selection are identified using the CODEML analysis
181 in PAML (Version 4.7; Yang 2007). For amino acids identified as under putative positive
182 selection, we imported their physiochemical properties, including hydrophobicity, steric bulk,
183 polarity, and electronic effects (Sandberg et al. 1998), into a matrix in Genesis 1.7.6 (Sturn et al.
184 2002). Finally, using hierarchical clustering via Euclidean distance methods, we identified
185 supertypes based on antigen binding similarity. The range of the number of alleles collapsed
186 into each supertype grouping is 1-8 alleles, with a mean ± S.D. of 2.01 ± 1.54 alleles.
187
188 *Genital secretion sample collection*
189 We collected secretion samples from the genital glands of 'donor' animals from two
190 captive 'populations': the DLC over 10 years (2003-2013, *N* = 24 females, 24 males) during the
191 breeding and nonbreeding seasons, and the Indianapolis Zoo (2011, *N* = 8 females, 1 male)
192 during the breeding season. For ring-tailed lemurs in the Northern Hemisphere, we considered
193 samples collected from November to March to be 'breeding season' samples and those
194 collected from May to August to be 'nonbreeding season' samples (Drea 2007, Scordato et al.
195 2007). At the DLC, trained handlers carefully caught and gently restrained the animals, which
196 were awake and habituated to these procedures. At the Indianapolis Zoo, collections occurred
197 during annual physical examinations, performed by Zoo staff members, while the animals were
198 under anesthesia. No secretions were collected from subjects at the Cincinnati Zoo. Following
199 published methods (Scordato et al. 2007), we used pre-cleaned cotton swabs and forceps to

collect the secretions. We placed the scented swabs in pre-cleaned chromatography vials and stored the vials at -80 °C until their use in chemical analysis or behavioral experiments. We have previously shown that individual-specific scent signatures are stable across both years and storage time (Scordato et al. 2007, Crawford et al. 2011, Drea et al. 2013). These odors were used for either chemical analyses or bioassay presentation, based upon the season of collection, the number of odors available per individual, and the number of possible recipients to which the odor could be presented. In order to maximize the possible bioassay presentations, we prioritized achieving an appropriate sample size for chemical analyses to detect statistical differences rather than analyzing the chemistry of every individual.

Gas chromatography-mass spectrometry

All chemical analyses were performed on secretions collected from subjects at the DLC. We used previously published methods to quantify the volatile chemical composition of a subset of these genital secretions (collected from *N* = 20 females, 23 males). These data include previously published data from some individuals (*N* = 17 females, 19 males; Scordato et al. 2007, Charpentier et al. 2008, Boulet et al. 2010), as well as unpublished data from additional individuals (N = 3 females, 5 males). Briefly, we extracted the volatile components of the secretions into 1.5 ml of methyl-*tert*-butyl ether, concentrated the extraction, and analyzed the components on a Shimadzu GCMS-QP2010 instrument (Shimadzu Scientific Instruments) equipped with a Shimadzu AOC-20 series autosampler. The compounds were detected using the automatic peak detector (SOLUTION WORKSTATION software, Shimadzu Scientific Instruments) and the peaks individually verified via consultation with the National Institute of Standards and Technology library (for further details, see Drea et al. 2013).

For analyses of the chemical data, we discarded compounds with inconsistent retention times or that did not comprise at least 0.05% of the overall area of the GC-MS chromatogram. The remaining compounds (*N* = 338 compounds from female genital secretions and 203

compounds from male genital secretions) consisted of fatty acids, fatty acid esters, cholesterol derivatives, alkanes, and other unidentified compounds (Scordato et al. 2007, Charpentier et al. 2008, Boulet et al. 2010). To represent the overall chemical composition of lemur genital secretions (Charpentier et al. 2008), we used three measures of diversity: richness, the Shannon index, and the Simpson index (Legendre & Legendre 1998, McCune et al. 2002). For our purposes, richness is the absolute number of compounds present per chromatogram regardless of relative abundance or rarity. By contrast, the Shannon and Simpson diversity indices reflect the relative abundances in different ways: The Shannon index is primarily influenced by common compounds of intermediate abundance, whereas the Simpson index gives more weight to compounds of the greatest relative abundance (McCune et al. 2002, Charpentier et al. 2008). We calculated these diversity indices for each individual's overall chemical profile, as well for specific subsets of compounds that have been implicated in signaling fertility: fatty acids (*N* = 33/338 and 25/203 in female and male genital secretions, respectively) and fatty acid esters (*N* = 112/338 and 87/203; Boulet et al. 2010, Crawford et al. 2011).

Behavioral bioassays

To test if ring-tailed lemur 'recipients' can use the secretions of 'donors' to discriminate between the MHC genotypes of opposite-sex conspecifics, we conducted 300 behavioral trials or 'bioassays,' (Scordato et al. 2007, Charpentier et al. 2008, Greene et al. 2016). To maximize our use of 'unknown' donors per recipient (i.e., the recipient had never resided in a group with the donor nor had smelled secretions from the donor during a previous bioassay experiment), we used recipients (*N* = 27) from the multiple institutions, including at the DLC (*N* = 5 females and 14 males), Cincinnati Zoo (*N* = 2 females), and Indianapolis Zoo (*N* = 2 females and 4 males), and secretion samples from 'unknown' donors of the opposite sex at the DLC (*N* = 31 females and 18 males). Bioassays were conducted from November-February in 2011 and 2012 following

252 previously established protocols (see Scordato et al. 2007, Charpentier et al. 2008, Greene et al.
253 2016). First, we temporarily isolated a recipient from its group members, then secured three fresh
254 wooden dowels in a row to the fence of the animal's enclosure (separated by 20 cm) at a 45° angle
255 to the ground. The center dowel served as an unscented control, whereas a ~2 cm area of the
256 outer dowels (at lemur nose level) were rubbed for ~10-15 seconds with the scented swabs from
257 different donors, to simulate a naturally placed scent mark. Each recipient underwent 1-3 trials per
258 day over 4-6 days, with each trial lasting 10 minutes, ultimately participating in 8-12 trials in total.
259 We presented the secretions to each recipient in randomized order. We also maximiated the
260 number of donor dyads whose secretions could be presented across recipients, while minimizing
261 the number of times we presented a given donor's secretions to any recipient (average ± S.D.
262 exposures = 1.85 ± 1.05, range = 0-6). Upon completion of the day's trials, the recipient was
263 reunited with its group.
264 The bioassays were videotaped and the videos were scored by three observers, using an
265 established ethogram. Prior to scoring experimental trials, we calculated inter-observer reliability
266 from five 'practice' trials. Differences in the labeling of an event or in the chronology or timing
267 (>1 s) were considered disagreements (Martin & Bateson 2007, Scordato et al. 2007) and
268 scoring of videos did not commence until inter-observer reliability scores exceeded 90%. The
269 behavior recorded included the recipient's proximity to each dowel and its sniffing, licking, biting,
270 genital marking and, for males only, shoulder rubbing, wrist marking, and tail rubbing
271 (Supplemental Table 2, adapted from Scordato et al. 2007). We also recorded where
272 investigatory or scent-marking behavior occurred relative to each scent 'mark' (i.e., whether the
273 behavior was directed at the mark itself, adjacent to the mark, but on the dowel, or within 15 cm
274 of the dowel). Specifically, we recorded the location of countermarks because their placement
275 could have particular significance: Overmarking or placing one's mark directly on top of the original
276 mark might mask the original mark, whereas adjacent-marking or placing one's mark near the
277 original mark leaves the original mark intact (Drea 2014).

278

279 *Statistical Analyses*

280

281 *General analytical procedures*

282 To examine the relationships between MHC-DRB genotype and olfactory ornamentation,
283 as well as the ability of ring-tailed lemurs to discriminate MHC genotype via genital secretion,
284 we analyzed the data in a series of generalized linear mixed models (GLMMs), using the
285 package 'glmmADMB' (Version 0.7.7) in RStudio (Version 3.0.2; RStudio 2015). MHC diversity
286 can be measured in several ways (e.g. the number of alleles in an individual's genotype, the
287 number of supertypes, or the number of amino acid differences between all alleles in an
288 individual's genotype, described below), as can MHC similarity between individuals. We
289 assessed several measures of MHC diversity for an individual, as well as several measures of
290 similarity between dyads. Because we expected all measures of MHC genetic diversity to be
291 correlated, we evaluated each of the explanatory genetic variables independently, and used
292 Akaike information criteria (AIC) values to determine the best model (Zuur et al. 2009). We
293 considered the model with the lowest AIC value to be the best fit for the data and report only
294 those models. To examine if MHC similarity were reflected in chemical similarity, we used partial
295 Mantel tests to compare the number of un-shared or unique MHC-DRB supertypes to the
296 relative Euclidean distance matrices between male-male (MM), female-female (FF), and male-
297 female (MF) dyads.

298

299 *Analyses of MHC-DRB diversity and chemical complexity in individual males and females*

300 To examine the relationship between MHC-DRB diversity and chemical complexity, we
301 analyzed the sexes separately and each chemical diversity index was evaluated in a separate
302 series of GLMMs using either a Gaussian or gamma distribution (see Supplemental Table 3).
303 We included donor identity as a random variable. Explanatory variables included season (i.e.,

304 breeding and nonbreeding) and one of four different genetic variables. Our four measures of
305 MHC-DRB diversity were 1) the absolute number of MHC-DRB alleles (MHC_{allele}), 2) the number
306 of MHC-DRB supertypes ($MHC_{supertype}$), 3) the average nucleotide base-pair differences between
307 all alleles within an individual donor ($MHC_{seqmean}$), and 4) the mean number of amino acid
308 differences between the alleles possessed by an individual donor (MHC_{aamean}; Supplemental
309 Table 3). Because of the skew in frequency of specific MHC-DRB supertypes, i.e., seven
310 supertypes were found in fewer than five individuals whereas one supertype was found in more
311 than 85% of individuals, we were unable to examine if the possession of a specific supertype
312 can be signaled via the chemical complexity of genital secretions. For females, we also
313 analyzed the diversity of two subsets of chemicals, fatty acids (FAs) that are known to signal
314 fertility in female primates (Michael et al. 1974, Doty et al. 1975, Matsumoto-Oda et al. 2003),
315 and fatty acid esters (FAEs), which are synthesized from FAs (Cheng & Russell 2004, Hargrove
316 et al. 2004). We have shown that both chemical subsets are correlated with microsatellite
317 diversity of female ring-tailed lemurs during the breeding season (Boulet et al. 2010). We were
318 unable to control for neutral heterozygosity estimated via microsatellites (see Charpentier et al.
319 2008, Boulet et al. 2010 for microsatellite methods), because microsatellite data were
320 unavailable for > 20% of our subjects. Nevertheless, we did assess the correlation between
321 microsatellite heterozygosity and MHC diversity, i.e., the number of MHC-DRB alleles and
322 MHC-DRB supertypes. Using the subset of subjects for which both genetic measures of
323 diversity were available (N = 36), we found no correlation between microsatellite heterozygosity
324 and the number of MHC-DRB alleles within an individual (correlation coefficient = 1.056, F =
325 1.51, P = 0.227), and no correlation between microsatellite heterozygosity and the number of
326 MHC-DRB supertypes (correlation coefficient = -0.8812, F = 1.461, P = 0.235; however, see
327 Grogan et al. 2017). As we had previously found no correlation between individual chemical
328 diversity and adult age, month of collection within season, or DLC housing condition
329 (Charpentier et al. 2008), we did not include these co-variables in our analyses. Lastly, to

ensure that the few individuals with the most diverse MHC-DRB genotype were not driving any association between MHC-DRB diversity and chemical diversity, we re-ran the final GLMMs after removing the most diverse individuals from both the male (*N* = 1) and female analyses (*N* = 1).

Analysis of MHC relatedness and chemical similarity between all possible dyads

To investigate if chemical similarity between dyads reflects similarity in their MHC genotypes, we calculated matrices of genetic distances using the number of different MHC supertypes between each dyad, or the number of different putative antigen binding pocket configurations that are present in one individual but not the other. We then estimated chemical distances between pairs of individuals by analyzing all chemicals present in secretion profiles of males (*N* = 203 compounds; Charpentier et al. 2008), females (*N* = 338; Boulet et al. 2010), or in both sexes (*N* = 170; Boulet et al 2009). We calculated relative Euclidean distance matrices for same-sex (MM or FF) and mixed-sex (MF) dyads, respectively, using PC-ORD (version 7.0, McCune & Mefford 2016), and following published protocols (Charpentier et al. 2008, Boulet et al. 2009). We calculated matrices separately as follows: breeding season (22 males, *N* = 231 MM dyads; 17 females, *N* = 136 FF dyads; 39 males and females, *N* = 374 MF dyads); nonbreeding season (20 males, *N* = 190 MM dyads; 18 females, *N* = 153 FF dyads; 38 males and females, *N* = 360 MF dyads). Because MM and FF matrices were square, we assessed linear relationships between chemical and MHC distances using partial Mantel tests in FSTAT (version 2.9.3.2, with 10,000 randomizations; Goudet 2001). As in previous studies (Charpentier et al. 2008, Boulet et al. 2010), we controlled for potentially confounding covariates, including the subject's age, social housing condition, and the month of secretion sample collection. For the MF comparisons, we first generated full matrices using all possible MM, FF, and MF pairs (breeding season *N* = 704 dyads, nonbreeding season *N* = 741 dyads). We then extracted chemical, genetic, and covariate information for MF dyads only. Unlike MM and FF matrices, the

356 MF matrix was not square. Therefore, we assessed relationships with 10,000 Spearman's
357 correlation permutation tests using the JMUOUTLIER package in R (Version 1.3; Garren 2016),
358 as in the study by Slade et al. (2016). Lastly, we assessed the correlation between MHC
359 similarity within dyads (i.e., the number of unique or unshared MHC-DRB alleles and supertypes
360 between two individuals) with dyad relatedness, as measured by the Queller and Goodnight
361 index (IDQG calculated by Boulet et al. 2010). We found that although dyad relatedness was
362 significantly correlated with MHC dissimilarity for both number of MHC-DRB alleles (*N* = 629
363 dyads, slope = -0.71, T-value = -4.21, P = 0.000029) and number of MHC-DRB supertypes (*N* =
364 629 dyads, slope = -0.67, T-value = -4.23, *P* = 0.000027), the negative relationships explained
365 less than 3% of the variance in either correlation (R^2 = 0.026 and R^2 = 0.026, respectively).
366
367 *Behavioral analyses of mixed-sex, recipient-donor combinations*
368 We explored the relationship between the recipients' behavioral responses to donor
369 secretions and measures of absolute and relative MHC-DRB diversity between the mixed-sex,
370 recipient-donor dyads (Table 1). To test for patterns that would be consistent with disassortative
371 mating under the good fit model, we used several measures of dissimilarity and sequence
372 divergence between each recipient-donor dyad (Schwensow et al. 2008, Setchell et al. 2010,
373 Huchard et al. 2013). We also used the donor's number of MHC alleles (MHC_{donor}) to examine if,
374 under the good genes model, the secretion of potential 'mates' with the greatest MHC diversity
375 were distinguished, regardless of their dissimilarity. Lastly, we also examined non-linear
376 relationships between MHC diversity and dissimilarity between dyads by including the quadratic
377 forms of all genetic explanatory variables in our GLMMs. Quadratic terms were retained only if
378 the AIC value was better than the GLMM that included only linear terms.
379 In our analyses, we excluded all recipient behavior that occurred in < 5% of trials and any
380 behavior that did not show a significant differential response via Wilcoxon signed-rank tests in
381 favor of the test dowels over the blank, control dowel (Supplemental Table 4; Charpentier et al.

2010). Ultimately, for male recipients, we analyzed proximity to the pole, sniffing and licking the mark and surrounding areas, shoulder rubs, and wrist marking the area adjacent to the mark. For females, we analyzed sniffing the mark and the adjacent area, as well as licking the mark. In each GLMM, we also included the non-genetic explanatory variables of trial number on a given day (i.e., 1-3), the number of times that a recipient had been presented with the secretion of a given donor over the course of the study (i.e., 1-6), as well as the secretion donor nested under secretion recipient, as a random term. For each behavioral response, we used either a Poisson or negative binomial distribution depending on the dispersion of the data (Supplemental Table 5) and applied a zero-inflation correction factor if appropriate. Initially, each GLMM was constructed using all explanatory terms, including both a linear and quadratic term for the genetic variable to account for potential nonlinear relationships. The GLMM with the lowest AIC value was chosen for further exploration, for which we sequentially dropped explanatory terms, then compared these models using AIC values. We report only the best fit models for each response variable below.

RESULTS

Signaling of individual quality via odor-gene covariance

We found that both sexes of ring-tailed lemurs signaled individual MHC quality via genital secretion chemistry, although the correlates of signaling differed between males and females: Male MHC diversity was positively correlated with chemical diversity, regardless of season (Figure 1; Table 2). Removal of the most diverse male from the analyses still resulted in significant relationships between MHC-DRB diversity and Shannon diversity ($P = 0.011$), between MHC-DRB and season ($P = 0.007$), and a trend toward significance for the interaction between MHC-DRB diversity, Shannon diversity, and season ($P = 0.068$). By contrast, female MHC diversity was unrelated to overall chemical diversity in either season (Figure 2; Table 3);

nevertheless, it was negatively correlated to the diversity of two important subsets of chemicals, FAs and FAEs, during the nonbreeding season. Similarly, removal of the most diverse female from the analysis of overall Shannon diversity and MHC-DRB diversity results in no significant relationships. After removal of the same female from the analyses of the chemical subsets of fatty acids and fatty acid esters, the interaction between season and MHC-DRB diversity in relation to Shannon diversity of fatty acids is no longer statistically significant although still trending in the same direction ($P = 0.10$). The relationship between fatty acid ester Shannon index in females and season retains a trending relationship ($P = 0.053$) and MHC-DRB diversity ($P = 0.067$), although their interaction is not significant ($P = 0.161$).

Signaling of relatedness via dyadic, odor-gene covariance

In all same-sex lemur dyads, genital olfactory cues encoded information about MHC distance, but in a season-dependent fashion (Figure 3; Table 4). After controlling for covariates, chemical distances between MM dyads correlated with unique MHC supertypes during the breeding season ($P < 0.001$, Figure 3A-B), but not during the nonbreeding season ($P = 0.270$, Figure 3C-D). Similarly, for FF dyads, we observed significant, season-specific correlations between the number of unique MHC supertypes and chemical distances (breeding season $P < 0.001$, Figures 3E-F; nonbreeding season $P = 0.729$, Figure 3G-H).

Although we could not find any relationship between chemical distance and MHC distance between MF dyads in the breeding season ($r = 0.0014$, $P = 0.8280$; Figure 4A), but we did find a trending negative relationship between mixed-sex dyads during the nonbreeding season ($r = -0.0099$, $P = 0.0647$; Figure 4B).

Olfactory discrimination of MHC genotype between mixed-sex conspecifics

Although in the breeding season, we could only detect the chemical signaling of MHC diversity in males, both male (Table 5; Figure 5) and female (Figure 6) recipients showed significant behavioral discrimination between the genital secretions of opposite-sex, conspecific donors based on their possession of different MHC-DRB genotypes. The pattern of response to conspecific secretions, however, differed between the sexes.

Male recipients did not differ in the time they spent in proximity to either of the scented dowels, nor did they differ in the number of wrist marks they directed to the area adjacent the mark; however, they investigated female secretions more if the donors were more similar at the MHC-DRB to themselves than if the donors were more dissimilar at the MHC-DRB. Specifically, compared to their responses to the secretions of MHC-DRB dissimilar females, male recipients spent more time sniffing the secretions of MHC-DRB similar females, more time sniffing the area adjacent to the mark, and responded with more shoulder rubs (Table 5; Figure 5). Additionally, as the absolute MHC-DRB diversity of female donors increased, male recipients also licked the mark more frequently (Table 5). Because the frequency of occurrence and duration of behavior were correlated for licking, shoulder rubbing, and wrist marking, we report on only one measure (either frequency or duration) per behavior (Table 5; Figure 5).

Female recipient responses to the mark itself did not differ according to the MHC-DRB diversity of male donors; nevertheless, female recipients did direct differential responses towards the areas adjacent to the male's mark. Specifically, as the MHC-DRB amino acid dissimilarity of the male donor increased, female recipients spent more time sniffing areas adjacent to the mark (z value = 3.11, P = 0.002; Figure 6).

DISCUSSION

Owing to its role in survival and reproductive success, immunogenetic diversity is an important predictor of mate quality and preference and may be signaled via visual or chemical means. Our study provides support for the socially salient, chemical signaling of genetic quality

458 in a strepsirrhine primate, as would be necessary according to the good genes and good fit
459 paradigm for choosing a mate or social partner. Despite sex differences in the chemical
460 'indicators' of quality and their seasonal emergence, ring-tailed lemurs of both sexes signaled
461 their MHC diversity and dissimilarity to conspecifics via the volatile component of their genital
462 secretions. Moreover, both sexes were able to use these and potentially other olfactory cues to
463 discriminate relevant information about the MHC genotypes of opposite-sex conspecifics. These
464 results confirm the functional significance of our previous work showing detectable relationships
465 between chemical diversity and microsatellite diversity in both sexes (Charpentier et al. 2008,
466 Boulet et al. 2010, Charpentier et al. 2010). Our results also provide a foundation from which to
467 explore if ring-tailed lemurs actually choose mates according to the good genes and/or good fit
468 models using reproductive success data from wild populations.

469 More specifically, males appeared to signal both MHC-DRB diversity and microsatellite
470 diversity, or their good genes, in a similar fashion: Both measures of genetic diversity were
471 positively correlated with overall chemical diversity of genital secretions, although the
472 relationship with microsatellite diversity only emerged in the breeding season, whereas the MHC
473 odor-gene covariance emerged regardless of season (albeit more strongly in the breeding
474 season). Females, however, signaled their good genes, i.e., genetic diversity, via specific
475 chemicals, e.g., fatty acids and fatty acid esters. Previously, we had shown that females
476 signaled increased microsatellite diversity via a negative relationship with the diversity of fatty
477 acids during the breeding season. Here, we show that females signal MHC diversity via a
478 negative relationship with FA and FAE diversity, but in the nonbreeding season instead.

479 Potentially, females may be signaling different information depending on the season.
480 During the breeding season, signaling genome-wide microsatellite diversity and relatedness
481 may be critical to avoid inbreeding (Boulet et al. 2009, 2010). In contrast, signaling MHC-
482 specific diversity and health during the nonbreeding season might convey health and
483 competitive ability during periods of intense female-female competition (e.g., López-Idiáquez et

484 al. 2016). The months of pregnancy and lactation are energetically expensive for female ring-
485 tailed lemurs (O'Mara & Hickey 2014). Additionally, intragroup female competition for access to
486 food increases (Sauther 1993, Gould et al. 2003). During these social disputes, the killing of
487 vulnerable infants is a significant risk, and is committed by both sexes (Hood 1994, Jolly et al.
488 2000, Ichino 2005, Charpentier & Drea 2013, Kittler & Dietzel 2016). Signaling one's health and
489 vitality may reduce the likelihood of aggressive encounters that could lead to infanticide by
490 competing females (reviewed in Stockley & Campbell 2013).

491 Our results contrast the lack of odor-gene covariance found in mandrills (*Mandrillus*
492 *sphinx*), the only other primate in which a relationship between secretion chemistry and MHC
493 diversity has been investigated. In both male and female mandrills, MHC diversity was unrelated
494 with the chemical diversity of secretions obtained from the surface of the sternal gland (Setchell
495 et al. 2011). MHC information, however, may be signaled through aspects of their olfactory
496 signature that were not analyzed by the authors. For instance, just as female ring-tailed lemurs
497 signal MHC and microsatellite diversity through a subset of chemicals (i.e., fatty acids and fatty
498 acid esters; Boulet et al. 2010), so too might MHC-DRB information be contained in the
499 composition and relative abundance of a subset of the compounds present in certain secretions.
500 Alternatively, information may be encoded in the non-volatile portion of the secretion (Hurst et
501 al. 2001, Beynon et al. 2002, Brennan & Zufall 2006, Kwatra & Drea 2007) or be signaled
502 through the composition of the microbiota present in the gland (Boehm & Zufall 2006, Zomer et
503 al. 2009, Archie & Theis 2011, Pearce et al. 2017) and the odorants they produce (Gorman et
504 al. 1974, Theis et al. 2013). Further exploration of individual compounds, specific subsets of
505 chemicals, or the non-volatile fraction of the mandrill secretion might yield a signaling pattern
506 that conveys information about MHC genotype. Such evidence would support findings that male
507 mandrills appear to use the MHC genotype of a potential mate for mate guarding decisions
508 (Setchell et al. 2016) and that MHC diversity is correlated with male reproductive success
509 (Setchell et al. 2010).

510 The chemical composition of lemur genital secretions also signals MHC dissimilarity, or fit,
511 between male-male, female-female, and male-female dyads, echoing previous results
512 demonstrating the same pattern for microsatellite diversity (Charpentier et al. 2008, Boulet et al.
513 2009 & 2010). Signaling relatedness to any potential social 'partner' could be relevant
514 throughout the year, to avoid related competitors or beneficially direct nepotism (Charpentier et
515 al. 2008, 2010). Signaling relatedness or compatibility to opposite sex conspecifics would be
516 particularly important in the breeding season to avoid inbreeding and maximize offspring
517 diversity (Brown 1999, Tregenza & Wedell 2000, Neff & Pitcher 2005). Evidence now exists that
518 odorants signal MHC dissimilarity within same-sex and opposite-sex dyads in two taxa formerly
519 thought to be primarily visually oriented, namely birds (black-legged kittiwake: Leclaire et al.
520 2014; song sparrows: Slade et al. 2016) and anthropoid primates (mandrills: Setchell et al.
521 2011), suggesting greater relevance of olfactory cues than previously suspected.
522 Regarding behavior, our male recipients responded most to the scent of females that had
523 the most similar MHC-DRB genotypes to their own. Such a pattern may reflect 'preferences,'
524 indicating that males may be avoiding outbreeding depression (Sommer 2005b), seemingly
525 contrary to the good fit prediction that individuals should choose mates to maximize their
526 dissimilarity. Nevertheless, an increase in response time may not necessarily indicate an
527 increase in interest. Instead, increased male investigation could simply reflect that more
528 processing time was required to decipher the female's potential as a mate. Previously, in a
529 study of microsatellite diversity, we had shown that male ring-tailed lemurs spent more time
530 sniffing the secretions of less-related females (Charpentier et al. 2010). Regardless of the
531 potential contradiction, both sets of findings indicate that male ring-tailed lemurs are minimally
532 able to discriminate conspecifics according to both overall genetic relatedness and MHC
533 diversity/dissimilarity. Lastly, our finding that female ring-tailed lemurs spent the most time
534 sniffing the vicinity of secretions from MHC-DRB dissimilar males complements previous work

535 showing that females of other species show greater response to the scents of more MHC
536 dissimilar males than of more MHC-diverse males.
537 We have confirmed an honest olfactory mechanism of ornamentation and potential mate
538 choice, namely via genital odor-MHC gene covariance and discrimination, in both sexes of ring-
539 tailed lemurs. Likewise, information about immunogenetic quality and similarity may also
540 influence general social behavior, specifically for prioritizing agonistic or nepotistic interactions.
541 Female lemurs are expected to be choosy under the traditional paradigm of sexual selection
542 (Trivers 1972); however, mate choice may be equally important for male ring-tailed lemurs
543 (Parga 2006). In this species, females socially dominate their male conspecifics (Jolly 1966),
544 are strictly seasonal and generally fertile only 1-3 times per year for a period of less than 24
545 hours (Evans & Goy 1968, Van Horn & Resko 1977), and often cycle somewhat synchronously
546 with other females in the social group (Pereira 1991). Thus, both sexes should be choosy about
547 the competitive effort directed towards their potential partners. Our data extend the potential for
548 olfactory-based MHC discrimination across the primate order and add to a growing body of
549 literature suggesting that mate choice may depend on both MHC dissimilarity and diversity
550 (Kamiya et al. 2014).
551

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563

564 **Author Contributions**

565 CMD and KEG conceived of the idea for this study with help from MB & RLH. KEG produced
566 the MHC-DRB genotypes and conducted the behavioral bioassays, and MB & RLH produced
567 the secretion chemistry data. KEG, MB, & RLH performed the analyses and KEG wrote the
568 original draft of the manuscript. CMD critically revised the manuscript with assistance from KEG,
569 RLH, and MB. All authors have approved the final manuscript for publication.

570

571 **Data Accessibility**

572 CSV files of MHC, chemical, and behavioral data from bioassays, will be deposited into Dryad.
573 Analyses reported in this article can be reproduced using these data.

574

575 **Conflict of Interest**

576 The authors declare no conflict of interest.

577

578 **REFERENCES**

579 Aeschlimann PB, Haberli MA, Reusch TBH, Boehm T, & Milinski M. 2003. Female sticklebacks,
580 *Gasterosteus aculeatus,* use self-reference to optimize MHC allele number during mate
581 selection. *Behavioral Ecology and Sociobiology* 54: 119-126.

582 Andersson M. 1994. *Sexual Selection*. Princeton, New Jersey. Princeton University Press.

583 Aksenov AA, Gojova A, Zhao W, Morgan JT, Sankaran S, Sandrock CE, & Davis CE. 2012.
584 Characterization of Volatile Organic Compounds in Human Leukocyte Antigen Heterologous
585 Expression Systems: A Cell's "Chemical Odor Fingerprint." *ChemBioChem* 13: 1053–1059.

586 Archie E & Theis K. 2011. Animal behaviour meets microbial ecology. *Animal Behaviour* 82:
587 425-436.

588 Beynon RJ, Veggerby C, Payne CE, Robertson DHL, Gaskell SJ, Humphries RE, & Hurst JL.
589 2002. Polymorphism in major urinary proteins: molecular heterogeneity in a wild mouse
590 population. *Journal of Chemical Ecology* 28 (7): 1429-1446.

591 Blaustein AR. 1981. Sexual selection and mammalian olfaction. *The American Naturalist* 117:
592 1006-1010.

593 Boehm T & Zufall F. 2006. MHC peptides and the sensory evaluation of genotype. *Trends in*
594 *Neurosciences* 29: 100-107.

595 Bonneaud C, Chastel O, Federici P, Westerdahl H, & Sorci G. 2006. Complex Mhc-based mate
596 choice in a wild passerine. *Proceedings of the Royal Society of London, B Biological*
597 *Sciences* 273: 1111-1116.

598 Boulet M, Charpentier MJE, & Drea CM. 2009. Decoding an olfactory mechanism of kin
599 recognition and inbreeding avoidance in a primate. *BMC Evolutionary Biology* 9 (1): 281-
600 292.

601 Boulet M, Crawford JC, Charpentier MJE, & Drea CM. 2010. Honest olfactory ornamentation in
602 a female-dominant primate. *Journal of Evolutionary Biology* 23: 1558-1563.

603 Brennan PA & Zufall F. 2006. Pheromonal communication in vertebrates. *Nature* 444: 308-315.

604 Brown JH, Jardetzky TS. Gorga JC, Stern LJ, Urban RG, Strominger JL, & Wiley DC. 1993.
605 Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1.
606 *Nature* 364(6432): 33–39.

607 Brown JL. 1999. The new heterozygosity theory of mate choice and the MHC. *Genetica* 104:
608 215-221.

609 Burger D, Dolivo G, Marti E, Sieme H, & Wedekind C. 2015. Female major histocompatibility
610 complex type affects male testosterone levels and sperm number in the horse (*Equus*
611 *caballus*). *Proceedings of the Royal Society of London, B Biological Sciences* 282:
612 20150407.

613 Charpentier MJE, Boulet M, & Drea CM. 2008. Smelling right: the scent of male lemurs
614 advertises genetic quality and relatedness. *Molecular Ecology* 17: 3225-3233.

615 Charpentier MJE, Crawford JC, Boulet M, & Drea CM. 2010. Message 'scent': lemurs detect the
616 genetic relatedness and quality of conspecifics via olfactory cues. *Animal Behaviour* 80:
617 101-108.

618 Charpentier MJE & Drea CM. 2013. Victims of infanticide and conspecific bite wounding in a
619 female-dominant primate: a long-term study. *PloS One* 8: e82830.

620 Cheng JB & Russell DW. 2004. Mammalian wax biosynthesis II. Expression cloning of wax
621 synthase cDNAs encoding a member of the acyltransferase enzyme family. *Journal of*
622 *Biological Chemistry* 279: 37798-37807.

623 Crawford JC, Boulet M, & Drea CM. 2011. Smelling wrong: hormonal contraception in lemurs
624 alters critical female odour cues. *Proceedings of the Royal Society of London, B Biological*
625 *Sciences* 278 (1702): 122-130.

626 Crawford J & Drea CM. 2015. Baby on board: olfactory cues indicate pregnancy and fetal sex in
627 a non-human primate. *Biology Letters* 11(2): 20140831.

628 Ditchkoff SS, Lochmiller RL, Masters RE, Hoofer SR, & Van Den Bussche RA. 2001. Major
629 histocompatibility complex associated variation in secondary sexual traits of white-tailed
630 deer (*Odocoileus virginianus*): evidence for good genes advertisement. *Evolution* 55: 616-
631 625.

632 Doty RL, Ford M, Preti G, & Huggins GR. 1975. Changes in intensity and pleasantness of
633 human vaginal odors during menstrual-cycle. *Science* 190: 1316-1318.

634 Doytchinova IA & Flower D. 2005. In silico identification of supertypes for class II MHCs. The
635 *Journal of Immunology* 174: 7085–7095.

636 Drea CM. 2007. Sex and seasonal differences in aggression and steroid secretion in *Lemur*
637 *catta*: are socially dominant females hormonally 'masculinized'? *Hormones & Behavior* 51
638 (4): 555-567.

639 Drea CM. 2014. D'Scent of Man: A Comparative Survey of Primate Chemosignaling in Relation
640 to Sex. *Hormones and Behavior* 68: 117-133.

641 Drea CM, Boulet M, delBarco-Trillo J, Greene LK, Sacha CR, Goodwin, TE & Dubay GR. 2013.
642 The "Secret" in Secretions: Methodological Considerations in Deciphering Primate Olfactory
643 Communication. *American Journal of Primatology* 75 (7): 621-642.

644 Eizaguirre C, Yeates SE, Lenz TL, Kalbe M, & Milinski M. 2009. MHC-based mate choice
645 combines good genes and maintenance of MHC polymorphism. *Molecular Ecology* 18 (15):
646 3316-3329.

647 Ekblom R, Saether SA, Grahn M, Fiske P, Kålås JA, & Höglund J. 2004. Major
648 histocompatibility complex variation and mate choice in a lekking bird, the great snipe
649 (*Gallinago media*). *Molecular Ecology* 13: 3821-3828.

650 Evans CS & Goy RW. 1968. Social behaviour and reproductive cycles in captive ring-tailed
651 lemurs (*Lemur catta*). *Journal of Zoology* 156: 181-197.

652 Evans ML, Dionne M, Miller KM, & Bernatchez L. 2012. Mate choice for major histocompatibility
653 complex genetic divergence as a bet-hedging strategy in the Atlantic salmon (*Salmo salar*).
654 *Proceedings of the Royal Society of London, B Biological Sciences* 279: 379-386.

655 Forsberg LA, Dannewitz J, Petersson E, & Grahn M. 2007. Influence of genetic dissimilarity in
656 the reproductive success and mate choice of brown trout - females fishing for optimal MHC
657 dissimilarity. *Journal of Evolutionary Biology* 20: 1859-1869.

658 Freeman-Gallant CR, Meguerdichian M, Wheelwright NT, & Sollecito SV. 2003. Social pairing
659 and female mating fidelity predicted by restriction fragment length polymorphism similarity at
660 the major histocompatibility complex in a songbird. *Molecular Ecology* 12 (11): 3077–3083.

661 Garren S. 2016. jmuOutlier: Permutation tests for nonparametric statistics. R package version
662 1.1. See http://CRAN.R-project.org/package=jmuOutlier.

663 Gorman ML, Nedwell DB, & Smith RM. 1974. An analysis of the contents of the anal scent
664 pockets of *Herpestes auropunctatus* (Carnivora: Veverridae). *Journal of Zoology* 172: 389-
665 399.

666 Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices
667 (version 2.9.3). See http://www2.unil.ch/popgen/ softwares/fstat.html.

668 Gould L, Sussman RW, & Sauther ML. 2003. Demographic and life-history patterns in a
669 population of ring-tailed lemurs (*Lemur catta*) at Beza Mahafaly Reserve, Madagascar: A 15-
670 year perspective. *American Journal of Physical Anthropology* 120 (2): 182-194.

671 Greene LK, Grogan KE, Smyth KN, Adams CA, Klager SA, & Drea CM. 2016. Mix it and fix it:
672 functions of composite olfactory signals in ring-tailed lemurs. *Royal Society Open Science* 3:
673 160076.

674 Grogan KE. 2018. Data from: MHC genetic variation influences both olfactory signals and scent
675 discrimination in ring-tailed lemurs. *Behavioral Ecology*. Link: To be determined.

676 Grogan KE, McGinnis GJ, Sauther ML, Cuozzo FP, Drea CM. 2016. Next-generation
677 genotyping of hypervariable loci in many individuals of a non-model species: technical and
678 theoretical implications. *BMC Genomics* 17: 204.

679 Grogan KE, Sauther ML, Cuozzo FP & Drea CM. 2017. Genetic wealth, population health:
680 Major histocompatibility complex variation in captive and wild ring-tailed lemurs (*Lemur*
681 *catta*). *Ecology and Evolution* 7 (19): 7638-7649.

682 Hargrove JL, Greenspan P, & Hartle DK. 2004. Nutritional significance and metabolism of very
683 long chain fatty alcohols and acids from dietary waxes. *Experimental Biology and Medicine*
684 229(3): 215-226.

685 Havlicek J & Roberts SC. 2009. MHC-correlated mate choice in humans: a review.
686 *Psychoneuroendocrinology* 34(4): 497–512.

687 Hettyey A, Hegyi G, Puurtinen M, Hoi H, Török J, & Penn DJ. 2010. Mate Choice for Genetic
688 Benefits: Time to Put the Pieces Together. *Ethology* 116: 1-9.

689 Hood LC. 1994. Infanticide among ringtailed lemurs (*Lemur catta*) at Berenty Reserve,
690 Madagascar. *American Journal of Primatology* 33: 65-69.

691 Huchard E, Albrecht C, Schliehe-Diecks S, Baniel A, Roos C, Kappeler PM, & Brameier M.
692 2012. Large-scale MHC class II genotyping of a wild lemur population by next generation
693 sequencing. *Immunogenetics*, 64(12): 895–913.

694 Huchard E, Baniel A, Schliehe-Diecks S, & Kappeler PM. 2013. MHC-disassortative mate
695 choice and inbreeding avoidance in a solitary primate. *Molecular Ecology* 22: 4071-4086.

696 Hurst JL, Payne CE, Nevison CM, Marie AD, Humphries RE, Robertson DHL, Cavaggioni A, &
697 Beynon RJ. 2001. Individual recognition in mice mediated by major urinary proteins. *Nature*
698 414: 631-634.

699 Ichino S. 2005. Attacks on a wild infant ring-tailed lemur (*Lemur catta*) by immigrant males at
700 Berenty, Madagascar: Interpreting infanticide by males. *American Journal of Primatology* 67:
701 267-272.

702 Johansson BG & Jones TM. 2007. The role of chemical communication in mate choice.
703 *Biological reviews of the Cambridge Philosophical Society* 82: 265-89.

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

706 Jolly A, Caless S, Cavigelli S, Gould L, Pereira ME, Pitts A, Pride RE, Rabenandrasana HD,
707 Walker JD, & Zafison T. 2000. Infant killing, wounding and predation in *Eulemur* and *Lemur*.
708 *International Journal of Primatology* 21: 21-40.

709 Kaesler E, Kappeler PM, Brameier M, Demeler J, Kraus C, Rakotoniaina JH, Hämäläinen AM, &
710 Huchard E. 2017. Shared evolutionary origin of MHC polymorphism in sympatric lemurs.
711 *Molecular Ecology* 26(20): 5629-5645.

712 Kamiya T, O'Dwyer K, Westerdahl H, Senior A, & Nakagawa S. 2014. A quantitative review of
713 MHC-based mating preference: The role of diversity and dissimilarity. *Molecular Ecology* 23
714 (21): 5151–5163.

715 Kempenaers B. 2007. Mate Choice and Genetic Quality: A Review of the Heterozygosity
716 Theory. *Advances in the Study of Behavior* 37: 189-278.

717 Kittler K & Dietzel S. 2016. Female infanticide and female-directed lethal targeted aggression in
718 a group of ring-tailed lemurs (*Lemur catta*). *Primate Biology* 3 (2): 41–46.

719 Knapp LA, Robson J, & Waterhouse JS. 2006. Olfactory signals and the MHC: a review and a
720 case study in Lemur catta. *American Journal of Primatology* 68(6): 568–584.

721 Kromer J, Hummel T, Pietrowski D, Giani AS, SauterJ, Ehninger G, Schmidt AH, & Croy I. 2016.
722 Influence of HLA on human partnership and sexual satisfaction. *Scientific Reports* 6: 32550.

723 Kwak J, Willse A, Preti G, Yamazaki K, & Beauchamp GK. 2010. In search of the chemical
724 basis for MHC odourtypes. *Proceedings of the Royal Society of London, B Biological*
725 *Sciences* 277: 2417-2425.

Kwatra SG & Drea CM. 2007. *Proteomic analysis of ringtailed lemur scent gland secretions: glandular- and individual-specific protein profiles,* Poster presented at the 30th Meeting of the American Society of Primatologists. Winston-Salem, NC. (Abstract: *American Journal of Primatology* 69 Supplement 159: 108-109.

Landry C, Garant D, Duchesne P, & Bernatchez L. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London, B Biological Sciences* 268: 1279-1285.

Lanyon CV, Rushton SP, O'Donnell AG, Goodfellow M, Ward AC, Petrie M, Jensen SP, Gosling LM, & Penn DJ. 2007. Murine scent mark microbial communities are genetically determined. *FEMS Microbiology Ecology* 59: 576-583.

Leclaire S, van Dongen WFD, Voccia S, Merkling T, Ducamp C, Hatch SA, Blanchard P, Danchin E, & Wagner RH. 2014. Preen secretions encode information on MHC similarity in certain sex-dyads in a monogamous seabird. *Scientific Reports* 4: 6920.

Leclaire S, Jacob S, Greene LK, Dubay GR, & Drea CM. 2017a. Social odours covary with bacterial community in the anal secretions of wild meerkats. *Scientific Reports* 7.

Leclaire S, Strandh M, Mardon J, Westerdahl H, & Bonadonna F. 2017b. Odour-based discrimination of similarity at the major histocompatibility complex in birds. *Proceedings of the Royal Society of London, B Biological Science* 284: 20162466.

Legendre P & Legendre L. 1998. *Numerical Ecology*. Amsterdam, The Netherlands, Elsevier Science BV.

Leinders-Zufall T, Brennan P, Widmayer P, Chandramani P, Maul-Pavicic A, Jager M, Li X-H, Breer H, Zufall F, & Boehm T. 2004. MHC class 1 peptides as chemosensory signals in the vomeronasal organ. *Science* 306: 1033-1037.

López-Idiáquez D, Vergara P, Fargallo JA, & Martinez-Padilla J. 2016. Female plumage coloration signals status to conspecifics. *Animal Behaviour* 121: 101-106.

Martin P & Bateson P. 2007. *Measuring Behavior: An Introductory Guide*. New York, New York. Cambridge University Press.

Matsumoto-Oda A, Oda R, Hayashi Y Murakami H, Maeda N, Kumazakid K, Shimizud K, & Matsuzawa T. 2003. Vaginal fatty acids produced by chimpanzees during menstrual cycles. *International Journal of Primatology* 74: 75-79.

Mays HL & Hill GE. 2004. Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology and Evolution* 19: 554-559.

McCune B, Grace JB, & Urban DL. 2002. *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, OR.

McCune B & Mefford MJ. 2016. *PC-ORD. Multivariate Analysis of Ecological Data Version 7.* MjM Software Design, Gleneden Beach, OR.

762 Michael RP, Bonsall RW, & Warner P. 1974. Human vaginal secretions: volatile fatty acid
763 content. *Science* 186: 1217-1219.

764 Milinski M. 2014. Arms races, ornaments and fragrant genes: the dilemma of mate choice in
765 fishes. *Neuroscience & Biobehavioral Reviews* 46: 567-572.

766 Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, & Boehm T. 2005. Mate
767 choice decisions of stickleback females predictably modified by MHC peptide ligands.
768 *Proceedings of the National Academy of Sciences of the United States of America* 102:
769 4414-4418.

770 Neff BD & Pitcher TE. 2005. Genetic quality and sexual selection: an integrated framework for
771 good genes and compatible genes. *Molecular Ecology* 14: 19-38.

772 Neff BD, Garner SR, Heath JW, & Heath DD. 2008. The MHC and non-random mating in a
773 captive population of Chinook salmon. *Heredity* 101 (2): 175–185.

774 Ober C. 1999. Studies of HLA, fertility and mate choice in a human isolate. *Human*
775 *Reproduction Update* 5: 103–7.

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

779 Olsson M, Madsen T, Ujvari B, & Wapstra E. 2004. Fecundity and MHC affects ejaculation
780 tactics and paternity bias in sand lizards. *Evolution* 58 (4): 906-909.

781 O'Mara MT & Hickey CM. 2014. The development of sex differences in ring-tailed lemur feeding
782 ecology. *Behavioral Ecology and Sociobiology* 68: 1273-1286.

783 Parga JA. 2006. Male mate choice in *Lemur catta*. *International Journal of Primatology* 27 (1):
784 107-131.

785 Pearce DS, Hoover BA, Jennings S, Nevitt GA, & Docherty KM. 2017. Morphological and
786 genetic factors shape the microbiome of a seabird species (*Oceanodroma leucorhoa*) more
787 than environmental and social factors. *Microbiome* 5 (1): 146.

788 Pechouskova E, Dammhahn M, Brameier M, Fichtel C, Kappeler PM, & Huchard E. 2015. MHC
789 class II variation in a rare and ecological specialist mouse lemur reveals lower allelic
790 richness and contrasting selection patterns compared to a generalist and widespread
791 sympatric congener. *Immunogenetics*, *67* (4): 229–245

792 Penn DJ. 2002. The scent of genetic compatibility: sexual selection and the major
793 histocompatibility complex. *Ethology* 108: 1-21.

794 Penn D & Potts WK. 1998a. Chemical signals and parasite-mediated sexual selection. *Trends in*
795 *Ecology & Evolution* 13: 391-395.

796 Penn D & Potts W. 1998b. How do major histocompatibility complex genes influence odor and
797 mating preferences? *Advances in Immunology* 69: 411-436.

798 Penn DJ & Potts WK. 1999. The evolution of mating preferences and major histocompatibility
799 complex genes. *American Naturalist* 153: 145-164.

800 Piertney SB & Oliver MK. 2006. The evolutionary ecology of the major histocompatibility
801 complex. *Heredity* 96: 7-21.

802 Qvarnstrom A. 2001. Context-dependent genetic benefits from mate choice. *Trends in Ecology*
803 *& Evolution* 16: 5-7.

804 Radwan J, Tkacz A, & Kloch A. 2008. MHC and Preferences for Male Odour in the Bank Vole.
805 *Ethology* 114: 827-833.

806 Reusch TB, Haberli MA, Aeschlimann PB, & Milinski M. 2001. Female sticklebacks count alleles
807 in a strategy of sexual selection explaining MHC polymorphism. *Nature* 414: 300-302.

808 Richardson DS, Komdeur J, Burke T, & Von Schantz T. 2005. MHC-based patterns of social
809 and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society of*
810 *London, B Biological Sciences* 272: 759-767.

811 RStudio Team. 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. See
812 http://www.rstudio.com/.

813 Ruff JS, Nelson AC, Kubinak JL, & Potts WK. 2012. MHC Signaling during Social
814 Communication. Pgs 290-313. In: *Self and Nonself*. López-Larrea C (Eds). Springer, New
815 York, New York, USA.

816 Sandberg M, Eriksson L, Jonsson J, Sjöström M, & Wold S. 1998. New chemical descriptors
817 relevant for the design of biologically active peptides. A multivariate characterization of 87
818 amino acids. *Journal of Medicinal Chemistry* 41 (14): 2481–2491.

819 Sauther ML. 1993. The dynamics of feeding competition in wild populations of ringtailed lemurs
820 (*Lemur catta*). In: Kappeler PM & Ganzhorn JU (Eds.). *Lemur social systems and their*
821 *ecological basis*, pp. 135-152. New York, New York. Plenum.

822 Schwensow N, Fietz J, Dausmann K, & Sommer S. 2007. MHC-associated mating strategies
823 and the importance of overall genetic diversity in an obligate pair-living primate. *Evolutionary*
824 *Ecology* 22: 617-636.

825 Schwensow N, Eberle M, & Sommer S. 2008. Compatibility counts: MHC-associated mate
826 choice in a wild promiscuous primate. *Proceedings of the Royal Society of London, B*
827 *Biological Sciences* 275: 555-564.

828 Scordato ES & Drea CM. 2007. Scents and sensibility: Information content of olfactory signals in
829 the ringtailed lemur (*Lemur catta*). *Animal Behaviour* 73 (2): 301-314.

830 Scordato ES, Dubay G, & Drea CM. 2007. Chemical composition of scent marks in the
831 ringtailed lemur (*Lemur catta*): glandular differences, seasonal variation and individual
832 signatures. *Chemical Senses* 32: 493-504.

833 Setchell JM, Charpentier M, Abbott KA, Wickings EJ, & Knapp LA. 2009. Is brightest best?
834 Testing the Hamilton-Zuk hypothesis in mandrills. *International Journal of Primatology* 30:
835 825-844.

836 Setchell JM, Charpentier MJE, Abbott KM, Wickings EJ, & Knapp LA. 2010. Opposites attract:
837 MHC-associated mate choice in a polygynous primate. *Journal of Evolutionary Biology* 23
838 (1): 136-148.

839 Setchell JM, Vaglio S, Abbott KM, Moggi-Cecchi J, Boscaro F, Pieraccini G, & Knapp LA. 2011.
840 Odour signals major histocompatibility complex genotype in an Old World monkey.
841 *Proceedings of the Royal Society of London, B Biological Sciences* 278 (1703): 274-280.

842 Setchell JM, Richards SA, Abbott KM, & Knapp LA. 2016. Mate-guarding by male mandrills
843 (*Mandrillus sphinx*) is associated with female MHC genotype. *Behavioral Ecology* 27 (6):
844 1756-1766.

845 Singh PB, Brown RE, & Roser B. 1987. MHC antigens in urine as olfactory recognition cues.
846 *Nature* 327: 161-164.

847 Slade JWG, Watson MJ, Kelly TR, Gloor GB, & Bernards MA. 2016. Chemical composition of
848 preen wax reflects major histocompatibility complex similarity in songbirds. *Proceedings of*
849 *the Royal Society B: Biological Sciences* 283: 20161966.

850 Sommer S. 2005a. The importance of immune gene variability (MHC) in evolutionary ecology
851 and conservation. *Frontiers in Zoology* 2 (16).

852 Sommer S. 2005b. Major histocompatibility complex and mate choice in a monogamous rodent.
853 *Behavioral Ecology and Sociobiology* 58: 181-189.

854 Sommer S, Courtiol A, & Mazzoni CJ. 2013. MHC genotyping of non-model organisms using
855 next-generation sequencing: a new methodology to deal with artefacts and allelic dropout.
856 *BMC Genomics*, 14 (1): 542.

857 Stockley P & Campbell A. 2013. Female competition and aggression: interdisciplinary
858 perspectives. Philosophical Transactions of the Royal Society B 368: 20130073.

859 Sturn A, Quackenbush J, & Trajanoski Z. 2002. Genesis: cluster analysis of microarray data.
860 *Bioinformatics* 18 (11): 207-208.

861 Theis KR, Venkataraman A, Dycus JA, Koonter KD, & Schmitt-matzen EN. 2013. Symbiotic
862 bacteria appear to mediate hyena social odors. *Proceedings of the National Academy of*
863 *Sciences of the United States of America* 110 (49): 19832-19837.

864 Tregenza T & Wedell N. 2000. Genetic compatibility, mate choice and patterns of parentage.
865 *Molecular Ecology* 9: 1013-1027.

866 Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B (Ed.). *Sexual*
867 *Selection and the Descent of Man*, pp. 136-179. Chicago, Illinois. Aldine.

868 Van Horn RN & Resko JA. 1977. The reproductive cycle of the ringtailed lemur (*Lemur catta*):
869 sex steroid levels and sexual receptivity under controlled photoperiods. *Endocrinology* 101:
870 1579-1586.

871 Willse A, Belcher AM, Preti G, Wahl JH, Thresher M, Yang P, Yamazaki K, & Beauchamp GK.
872 2005. Identification of major histocompatibility complex-regulated body odorants by
873 statistical analysis of a comparative gas chromatography/mass spectrometry experiment.
874 *Analytical Chemistry* 77: 2348-2361.

875 Winternitz J, Abbate JL, Huchard E, Havlicek J, & Garamszegi LZ. 2016. Patterns of MHC-
876 dependent mate selection in humans and non-human primates: a meta-analysis. *Molecular*
877 *Ecology* 26 (2): 668-688.

878 Winternitz JC, Minchey SG, Garamszegi LZ, Huang S, Stephens PR, & Altizer S. 2013. Sexual
879 selection explains more functional variation in the mammalian major histocompatibility
880 complex than parasitism. *Proceedings of the Royal Society of London, B Biological*
881 *Sciences* 280: 20131605.

882 Yamazaki K, Boyse EA, Mike V, Thaler HT, Mathieson BJ, Abbott J, Boyse J, Zayas ZA, &
883 Thomas L. 1976. Control of mating preferences in mice by genes in the major
884 histocompatibility complex. *Journal of Experimental Medicine* 144: 1324-1335.

885 Yang Z. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. *Molecular Biology and*
886 *Evolution* 24: 1586–1591.

887 Ziegler A, Kentenich H, & Uchanska-Ziegler B. 2005. Female choice and the MHC. *Trends in*
888 *Immunology* 26: 496-502.

889 Zomer S, Dixon SJ, Xu Y, Jensen SP, Wang H, Lanyon CV, O'Donnell AG, Clare AS, Gosling
890 LM, Penn DJ, & Brereton RG. 2009. Consensus multivariate methods in gas
891 chromatography mass spectrometry and denaturing gradient gel electrophoresis: MHC-
892 congenic and other strains of mice can be classified according to the profiles of volatiles and
893 microflora in their scent-marks. *Analyst* 134: 114-123.

894 Zuur AF, Ieno EN, Walker NJ, Saveliev AA, & Smith GM. 2009. *Mixed Effects Models and*
895 *Extensions in Ecology with R*. New York, New York. Springer.

896

897 **Figure 1**. Seasonal relationships between chemical diversity in genital secretions and MHC
898 supertype diversity in male ring-tailed lemurs. Overall chemical diversity increased with
899 increasing MHC supertype diversity across the breeding season (A), shown in black circles, and
900 the nonbreeding season (B), shown in white circles.

901

902 **Figure 2**. Seasonal relationships between various measures of chemical diversity in genital
903 secretions and MHC supertype diversity in female ring-tailed lemurs. Overall chemical diversity
904 was not correlated with MHC supertype diversity in either the breeding season (A), shown in
905 black circles, or nonbreeding season (B), shown in white circles. Diversity indices for fatty acids
906 (FAs) and fatty acid esters (FAEs) were not correlated with MHC supertype diversity in the
907 breeding season (C and E, respectively), but were negatively correlated in the nonbreeding
908 season (D and F, respectively).

909

910 **Figure 3.** Relationships between genetic distance (number of unique MHC supertypes, i.e.,
911 $MHC_{supertype\ diff}$) and chemical distance (relative Euclidean) between male-male (A-D) and
912 female-female (E-H) ring-tailed lemur dyads during the breeding (closed circles; A-B and E-F)
913 and nonbreeding seasons (open circles; C-D and G-H). The numbers of dyads are provided in
914 the bottom panel.

915

916 **Figure 4.** Relationships between the number of unique MHC supertypes (i.e., $MHC_{supertype\ diff}$)
917 and chemical distance (relative Euclidean) between male-female, ring-tailed lemur dyads during
918 the breeding season (A-B), shown in black circles, and nonbreeding season (C-D), shown in
919 white circles. The numbers of dyads are provided in the bottom panel.

920

921 **Figure 5**. Response of male ring-tailed lemurs to the odorants of female conspecifics: As the
922 number of MHC-DRB supertypes between the male recipient and the female donor increases,
923 the duration of male sniffing (A) and the frequency of shoulder rubbing (B) decreases
924 significantly. As the diversity of the MHC genotype of the female donor increases, the duration
925 of licking (C) decreases. Points are jittered to avoid overlap of data.

926

927 **Figure 6.** Response of female ring-tailed lemurs to the odorants of male conspecifics: As the
928 number of amino acid differences between female recipient and male odorant donor increases,
929 the duration of time the female sniffs the male's odor increases.

930

931 **Table 1.** Explanatory variables used in the analysis of recipient responses to conspecific

932 odorants and the model under which they were tested.

Variable	Model	Definition
$MHC_{supertype\ diff}$	Good Fit	The number of MHC-DRB supertypes that are different between a recipient-donor dyad
$MHC_{allele\ diff}$	Good Fit	The number of MHC-DRB alleles that are different between a recipient-donor dyad
MHC_{min}	Good Fit	The minimum number of amino acid differences between a recipient-donor dyad's most similar MHC-DRB allele
MHC_{mean}	Good Fit	The mean number of pairwise amino acid differences between a recipient-donor dyad
$MHC_{aa\ diff}$	Good Fit	The total number of amino acid differences between all possible pairs of alleles between a recipient-donor dyad
MHC_{donor}	Good Genes	The number of MHC-DRB alleles present in the donor

933

934

935 **Table 2.** Relationships between measures of chemical diversity and of MHC diversity in male

936 ring-tailed lemurs across seasons, with significant relationships indicated in bold.

Measure of chemical diversity	Best-fit explanatory variable	Z value	*P* value	Effect
Richness-overall	Season	2.37	***0.018***	Chemical richness decreases from nonbreeding season to breeding season, but increases with increasing MHC diversity.
	$MHC_{supertype}$	2.31	***0.021***	
	Season * $MHC_{supertype}$	-0.71	*0.475*	
Shannon Index-overall	Season	2.43	***0.015***	Shannon diversity decreases from nonbreeding season to breeding season, but increases with increasing MHC diversity.
	$MHC_{supertype}$	2.52	***0.012***	
	Season * $MHC_{supertype}$	-1.45	*0.146*	
Simpson Index-overall	Season	1.64	*0.10*	Simpson diversity does not change from breeding to nonbreeding season; however, Simpson diversity increases with increasing MHC diversity.
	$MHC_{supertype}$	2.17	***0.03***	
	Season * $MHC_{supertype}$	-0.89	*0.37*	

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939 **Table 3.** Relationships between measures of chemical diversity and MHC diversity in female

940 ring-tailed lemurs across seasons, with significant relationships indicated in bold.

Measure of chemical diversity	Best-fit explanatory variable	Z value	*P* value	Effect
Richness-overall	Season	-0.53	*0.600*	No relationship between female richness and season or MHC diversity.
	$MHC_{supertype}$	-1.02	*0.310*	
	Season * $MHC_{supertype}$	-0.92	*0.360*	
Shannon Index-overall	Season	0.77	*0.442*	Overall Shannon index possibly negatively correlated with MHC diversity, but only in the nonbreeding season.
	$MHC_{supertype}$	-0.34	*0.733*	
	Season * $MHC_{supertype}$	-1.69	*0.091*	
Simpson Index-overall	Season	0.86	*0.390*	No relationship between female Simpson index and season or MHC diversity.
	$MHC_{supertype}$	0.24	*0.810*	
	Season * $MHC_{supertype}$	-0.98	*0.330*	
Shannon Index - FAs	Season	1.62	*0.104*	FA Shannon index negatively correlated with MHC diversity, but only in the nonbreeding season.
	$MHC_{supertype}$	-0.20	*0.838*	
	Season * $MHC_{supertype}$	-3.09	***0.002***	
Shannon Index - FAEs	Season	1.43	*0.152*	FAE Shannon index negatively correlated with MHC diversity, but only in the nonbreeding season.
	$MHC_{supertype}$	-0.42	*0.678*	
	Season * $MHC_{supertype}$	-2.63	***0.009***	

941

942

943 **Table 4**. Partial Mantel tests for same-sex (MM and FF) dyads, showing seasonal relationships
944 between genital odor distance (relative Euclidean) and MHC-based genetic distance in ring-
945 tailed lemurs. Odor distance is based on 203 and 338 compounds for MM and FF dyads,
946 respectively. Tests include three socio-demographic and environmental variables as covariates.
947 Significant ($P \leq 0.05$) sums of squares (SS), partial Mantel correlation coefficient (r), and P
948 values are shown in bold type, and trending ($P \leq 0.10$) values are shown in italics.

Dyad type	Variable	Number of unique MHC supertypes					
		Breeding season			Nonbreeding season		
		SS	*r*	*P*	SS	*r*	*P*
MM dyads	MHC	**2.309**	**0.408**	**<0.001**	0.042	-0.079	0.276
	Age	0.203	0.121	0.068	**0.139**	**0.145**	**0.005**
	Housing	0.157	0.106	0.101	0.006	-0.029	0.692
	Month of collection	0.006	-0.020	0.763	*0.124*	*0.137*	*0.062*
FF dyads	MHC	**0.391**	**0.313**	**<0.001**	0.003	0.027	0.738
	Age	0.001	0.014	0.872	0.008	-0.047	0.560
	Housing	0.029	0.085	0.323	0.004	0.034	0.679
	Month of collection	*0.093*	*0.153*	*0.076*	**1.461**	**0.623**	**<0.001**

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950

951 **Table 5.** Relationship between the MHC-DRB genotype of female odorant donors and male
952 recipient behavior, directed toward the female's scent mark, with significant relationships
953 indicated in bold. Explanatory variables with superscripts indicate the quadratic variable, while
954 those without superscripts are linear.

Behavior	Best-fit explanatory variable	Z value	*P* value	Effect
Sniff mark	$MHC_{supertype\ diff}^2$	-2.10	**0.036**	Shorter duration with increasing supertype differences between dyad
Sniff pole	$MHC_{supertype\ diff}^2$	-2.54	**0.011**	Shorter duration with increasing supertype differences between dyad
Lick mark	MHC_{donor}	-4.45	**<0.001**	Shorter duration with increasing female donor MHC diversity
	MHC_{donor}^2	4.14	**<0.001**	
Shoulder rub	$MHC_{supertype\ diff}^2$	-2.09	**0.037**	Fewer rubs with increasing supertype differences

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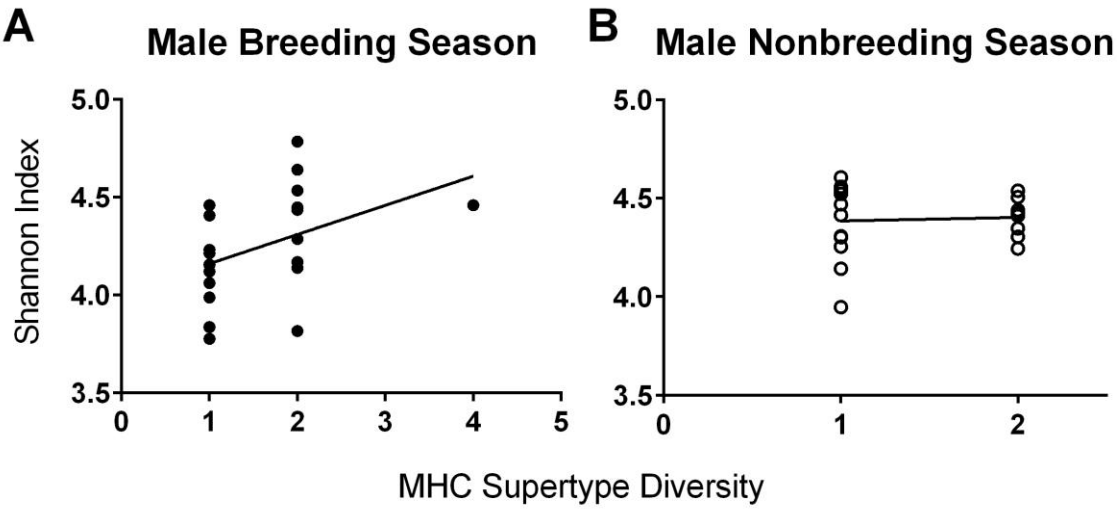
957 **Figure 1**.



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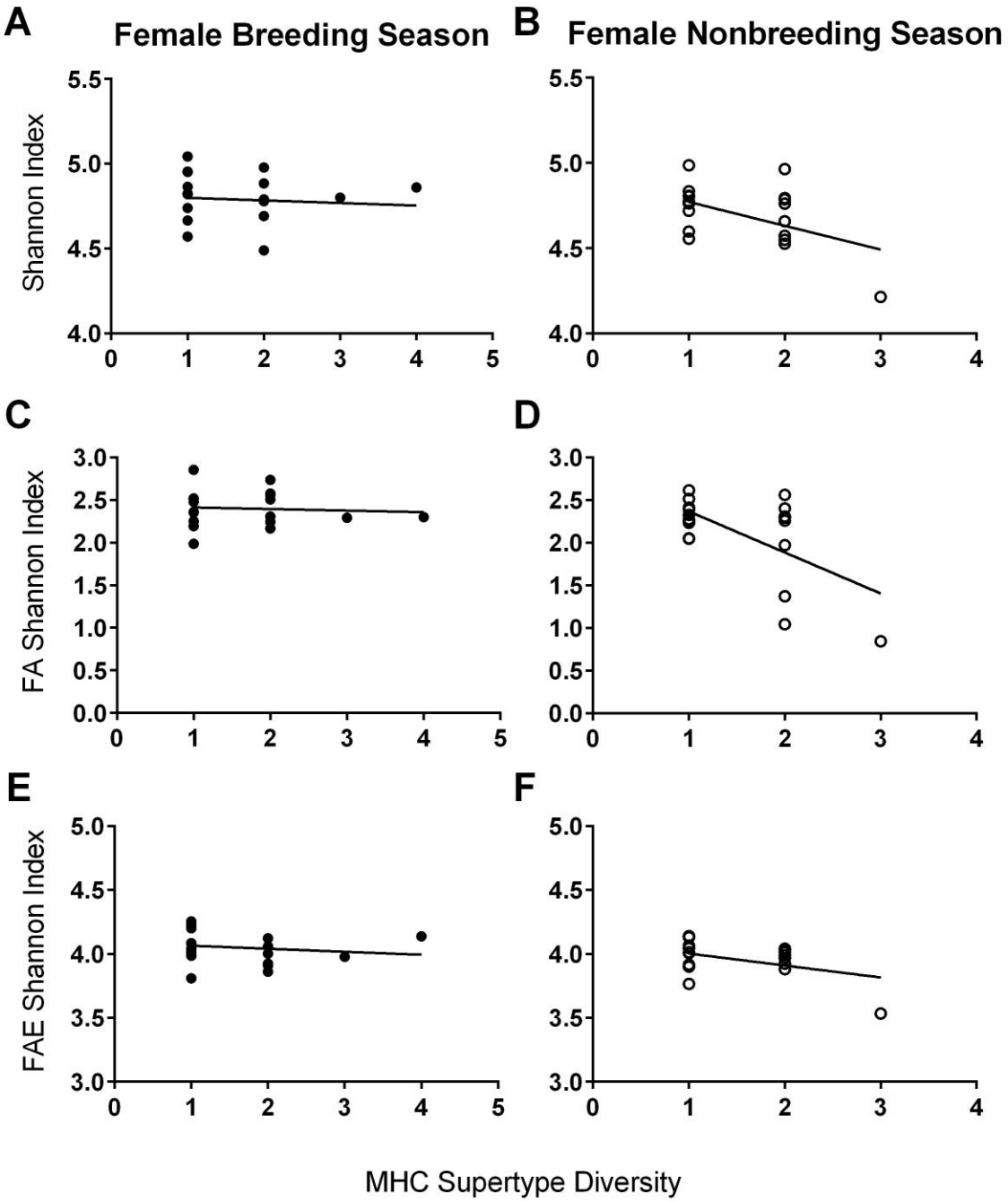
960 **Figure 2**.



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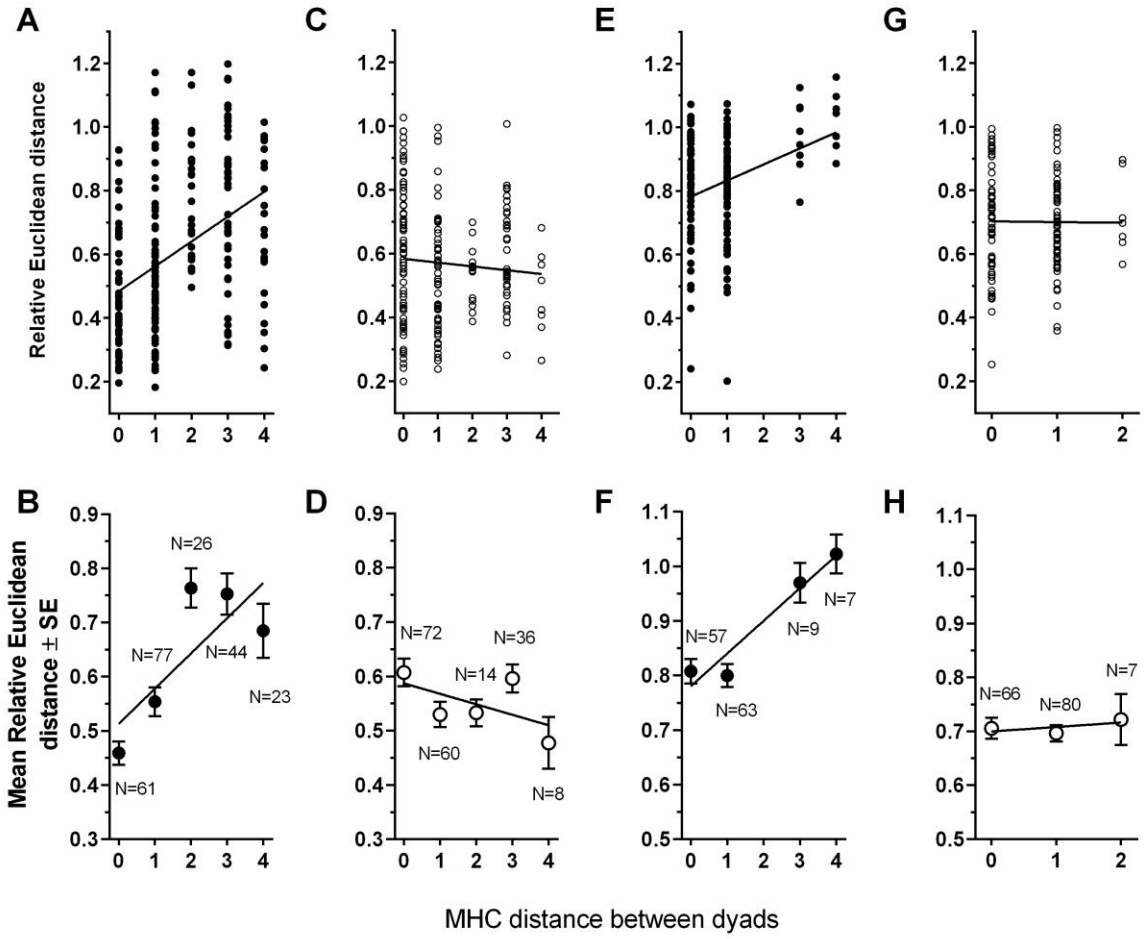
963 **Figure 3.**



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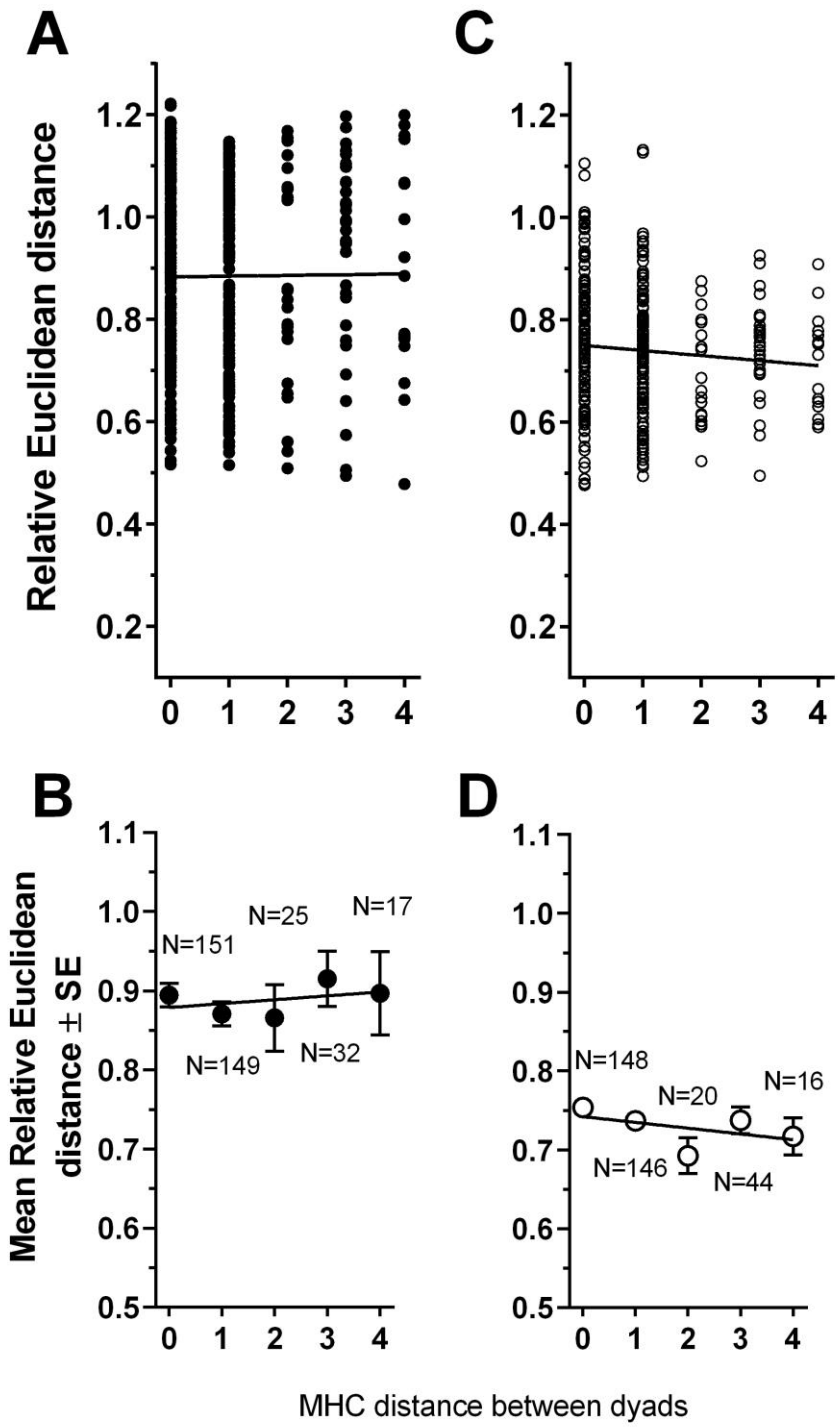
966 **Figure 4.**



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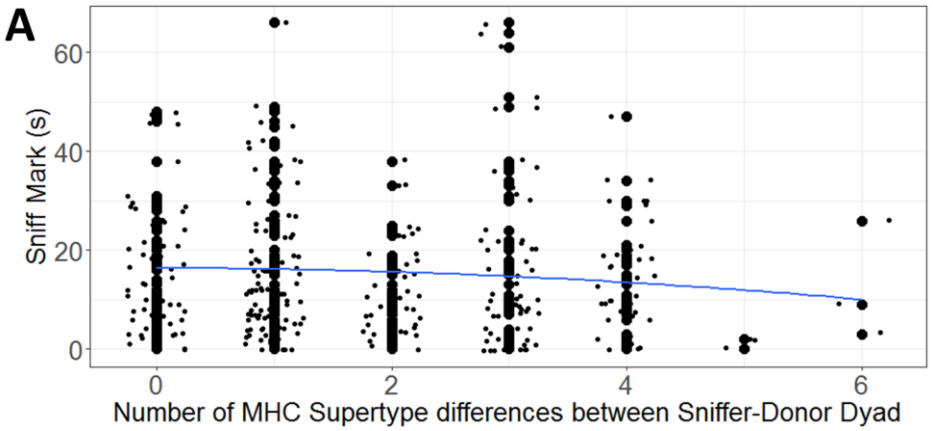
969 **Figure 5**.



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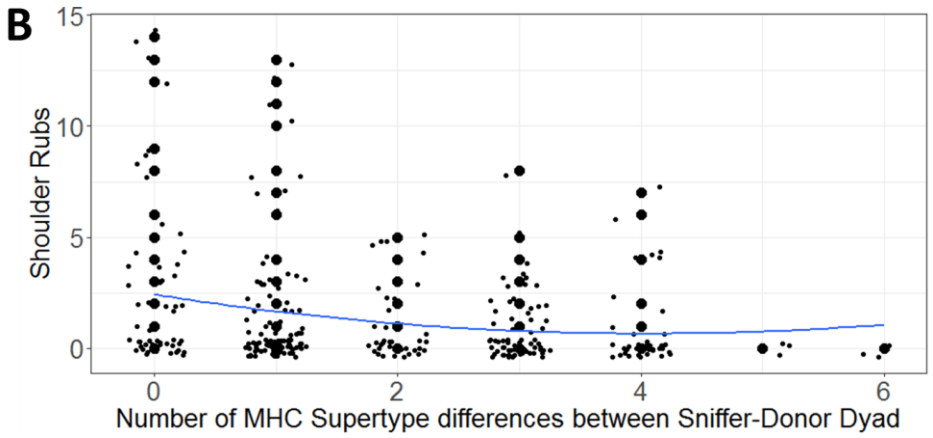

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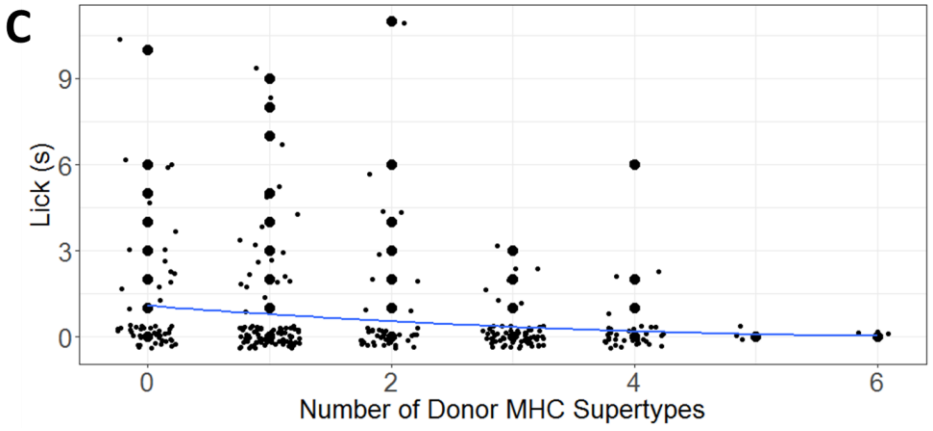

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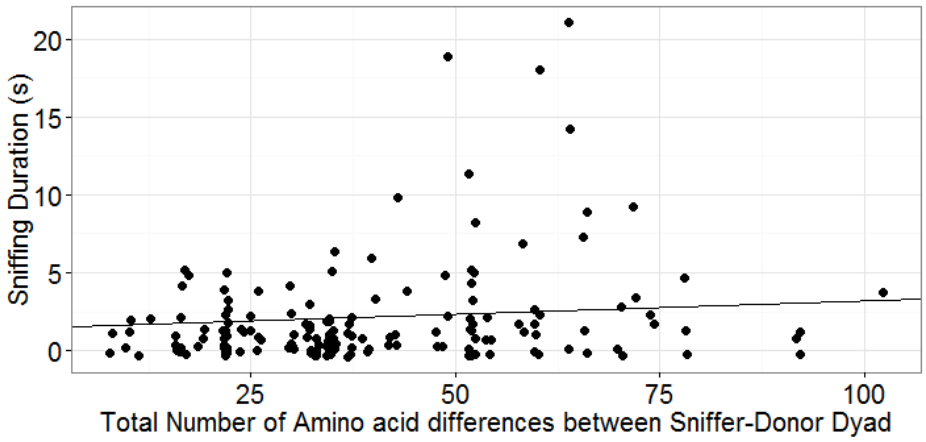
974 **Figure 6.**



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