BEHAVIORAL ECOLOGY

Mandrills use olfaction to socially avoid parasitized conspecifics

Clémence Poirotte,[1,2] François Massol,[3] Anaïs Herbert,[4] Eric Willaume,[5] Pacelle M. Bomo,[5] Peter M. Kappeler,[6] Marie J. E. Charpentier[1,2]*

The evolutionary transition from a solitary to a social lifestyle entails an elevated parasite cost because the social proximity associated with group living favors parasite transmission. Despite this cost, sociality is widespread in a large range of taxonomic groups. In this context, hosts would be expected to have evolved behavioral mechanisms to reduce the risk of parasite infection. Few empirical studies have focused on the influence of pathogen-mediated selection on the evolution of antiparasitic behavior in wild vertebrates. We report an adaptive functional relationship between parasitism and social behavior in mandrills, associated with evidence that they are able to gauge parasite status of their group members. Using long-term observations, controlled experiments, and chemical analyses, we show that (i) wild mandrills avoid grooming conspecifics infected with orofecally transmitted parasites; (ii) mandrills receive significantly more grooming after treatment that targets these parasites; (iii) parasitism influences the host's fecal odors; and (iv) mandrills selectively avoid fecal material from parasitized conspecifics. These behavioral adaptations reveal that selecting safe social partners may help primates to cope with parasite-mediated costs of sociality and that "behavioral immunity" plays a crucial role in the coevolutionary dynamics between hosts and their parasites.

INTRODUCTION

Parasites act as selective agents by imposing substantial costs on their hosts, affecting species both at individual and population levels (*1*, *2*). They potentially drive movement patterns (*3*), affect various life history traits, and may markedly affect the evolution of social life (*4*, *5*). Social species face high risk of infection because group living generates fertile ground for parasite transmission (*6*, *7*). Therefore, parasite-mediated selection has driven the emergence of complex host defense mechanisms to limit the spread of parasites (*8*–*10*). In addition to their physiological immune system, animals have developed a "behavioral immune system," which comprises a sophisticated set of parasite avoidance strategies, such as sanitary behaviors (*11*–*13*), that represent a first line of defense in decreasing parasite encounter rates.

Parasites may also influence host social relationships because conspecifics themselves represent a high risk of contagion. However, in animal societies, social avoidance of parasitism has been studied almost exclusively in the contexts of sexual selection (*14*) and kin selection in eusocial species, with reports of self-exclusion of sick animals from the social group (*15*). We propose that avoidance of parasitized individuals may occur in a much broader social context. Despite the growing acceptance of the idea that parasites may interfere with host behavioral interactions (*8*, *16*), data on parasitism as an evolutionary driver of host social relationships in wild vertebrate populations are lacking. Here, we study the influence of intestinal protozoa, transmitted from host to host by body contact via the orofecal route, on the behavioral strategies of wild mandrills living in southern Gabon.

Mandrills are group-living Old World monkeys living in dense equatorial rainforests and facing intensive parasite pressures in their natural habitat (*17*). As in other primates, allogrooming is a highly valuable social tool, buffering social competition induced by group living and enhancing the well-being and fitness of both groomer and groomee (*18*). Moreover, allogrooming is possibly an important hygienic behavior (*19*). However, intimate physical interactions between grooming partners, including the ingestion of particles, such as skin fragments and ectoparasites, should also favor orofecal transmission of protozoa. Here, we examined in a large free-ranging population of mandrills whether (i) parasitized individuals harbor protozoans on their fur, (ii) mandrills show patterns of allogrooming that could indicate that they are avoiding conspecifics with parasitic infections, and (iii) individuals discriminate conspecifics infected with protozoans by the smell of their feces.

We carried out coprological analyses of skin smears (one from the perianal area and one from other parts of the body) from 36 individuals of known parasite status. The proportion of smears with infectious protozoan stages was always higher in parasitized than in nonparasitized animals ($P \leq 0.05$ for three of the five protozoa taxa infecting these particular samples), and the proportion of positive smears was also always higher when collected around the perianal area than other body parts in parasitized animals ($P \leq 0.1$ for three of the four protozoa taxa infecting these particular samples) (table S1). Therefore, grooming parasitized conspecifics is potentially more risky for the groomer than when grooming nonparasitized conspecifics, leading to the prediction that individuals (i) should avoid grooming parasitized, contagious individuals and (ii) should particularly avoid grooming the perianal area of these individuals.

RESULTS AND DISCUSSION

For 2.5 years (October 2012 to April 2015), we performed behavioral observations using focal sampling on 25 mandrills from the studied population (13 females aged 4 to 22 years and 12 males aged 1.5 to 15 years) with known protozoan status (Supplementary Materials). We calculated monthly individual indices of the time spent receiving or giving grooming. We analyzed these indices in relation to the monthly individual protozoan richness (mean number of taxa per individual per month) and the specific protozoan status for each studied taxon (mean number of fecal samples with a particular protozoan taxon per individual per month) (*17*).

[1]Centre d'Ecologie Fonctionnelle et Evolutive (CEFE)–CNRS, UMR 5175, 1919 Route de Mende, 34293 Montpellier Cedex 5, France. [2]Institut des Sciences de l'Evolution de Montpellier (ISEM), UMR 5554, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France. [3]CNRS, Université de Lille–Sciences et Technologies, UMR 8198, Evo-Eco-Paléo, F-59655 Villeneuve d'Ascq, France. [4]Centre de Primatologie, Centre International de Recherches Médicales de Franceville (CIRMF), BP 769, Franceville, Gabon. [5]Projet Mandrillus, Société d'exploitation du Parc de Lékédi (SODEPAL), BP 52, Bakoumba, Gabon. [6]Behavioral Ecology and Sociobiology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany.
*Corresponding author. Email: marie.charpentier@cefe.cnrs.fr

We performed general linear mixed models (LMM) controlling for other confounding predictors (individual's sex, age, and social rank as fixed effects and individual identity as a random effect) and used a multimodel approach to show that the best models for grooming received, but not for grooming given, include protozoan richness [n = 105 individual.month (one individual sampled in a particular month)] (Table 1A). We considered that a predictor was plausible when its importance score, that is, the sum of the Akaike weights (*20*) of models incorporating this predictor, was higher than the expected value ("expected ratio") under the assumption that all tested models have equal Akaike weights. In agreement with our first prediction, individuals parasitized by a large number of protozoan taxa received less grooming from their conspecifics than individuals bearing low protozoan richness (Table 1A). When we performed models with a specific parasite status for the seven studied protozoan taxa, instead of richness, we found that only *Balantidium coli* decreased the grooming received in parasitized individuals (importance, 0.53; expected ratio, 0.50; estimate, −0.33) (table S2). However, the best model that includes protozoan richness had a lower Akaike information criterion (AIC) than the best model that includes *B. coli* (190.51 versus 196.79, respectively). Because no such relationships were observed between the grooming given and parasitism (Table 1B), the direction of allogrooming appears to be crucial regarding protozoan transmission: The observed effect is not simply due to spatial exclusion or self-isolation of parasitized individuals but is due to selective grooming from social partners. In line with this result, we further found that fine-grained spatial associations among a subset of 13 individuals, equipped with proximity data loggers for a year, were independent of their protozoan status (multimodel LMM approach). Several properties of the generated spatial networks (daily degrees and numbers of contact) were independent of an individual's protozoan richness (importance < expected ratio) (table S3).

Mandrills allocate, on average, 9.2% of their grooming time to the perianal area of their conspecifics. We therefore analyzed whether the protozoan status of the groomee predicted the probability that a received grooming event included the perianal area. Using generalized linear mixed models (GLMM) with a binomial distribution, we found that individuals parasitized by a large number of protozoan taxa received fewer grooming events involving the perianal area than individuals harboring few protozoan taxa (n = 31 individual.month, multimodel GLMM approach) (Table 1C), in accordance with our second prediction.

To further test our finding that individuals parasitized by a large number of protozoan taxa received less grooming, we deparasitized 16 individuals against protozoa either during regular observations (n = 3 individuals) or during captures (n = 13 individuals). We showed that the frequency of grooming received, but not of grooming given, significantly increased after treatment (Wilcoxon signed-rank tests, $P < 0.01$ and $P = 0.14$ resp.) (Fig. 1), whereas grooming received and given did not significantly increase for the nine control individuals that were captured but not deparasitized (Wilcoxon signed-rank tests, $P > 0.1$ in both instances) (fig. S1).

Using both correlative and experimental approaches, we document parasite-induced behavioral plasticity, suggesting that social avoidance behavior evolved in this Old World primate as a host strategy to limit

Table 1. Effects of predictors on the index of (A) grooming received and (B) grooming given and on (C) the proportion of grooming events, including the perianal area. For each predictor, we calculated the sum of Akaike weights of the models, including the predictor (importance) and compared it to the expected value under the assumption that all tested models have equal Akaike weights (expected ratio) to show the plausibility of each predictor. Plausible predictors (in bold) are those with an importance greater than the expected ratio. The estimate coefficient associated to each predictor variable and its SEM are reported. Dominance in interaction with sex is not shown in the tables because this interaction was never included in the set of best models.

Predictors	Importance	Expected ratio	Estimate	SEM
A				
Age	**0.99**	**0.5**	**0.05**	**0.01**
Protozoan richness	**0.6**	**0.5**	**−0.10**	**0.06**
Sex	**0.53**	**0.33**		
Female			**0.02**	**0.27**
Male			**0.32**	**0.27**
B				
Sex	**0.98**	**0.33**		
Female			**0.77**	**0.13**
Male			**0.02**	**0.12**
Age	0.3	0.5	0.01	0.01
Protozoan richness	0.25	0.5	0.01	0.04
C				
Protozoan richness	**0.86**	**0.5**	**−0.84**	**0.37**
Age	0.45	0.5	0.15	0.11
Sex	0.17	0.5		
Female			1.31	2.13
Male			0.81	2.33

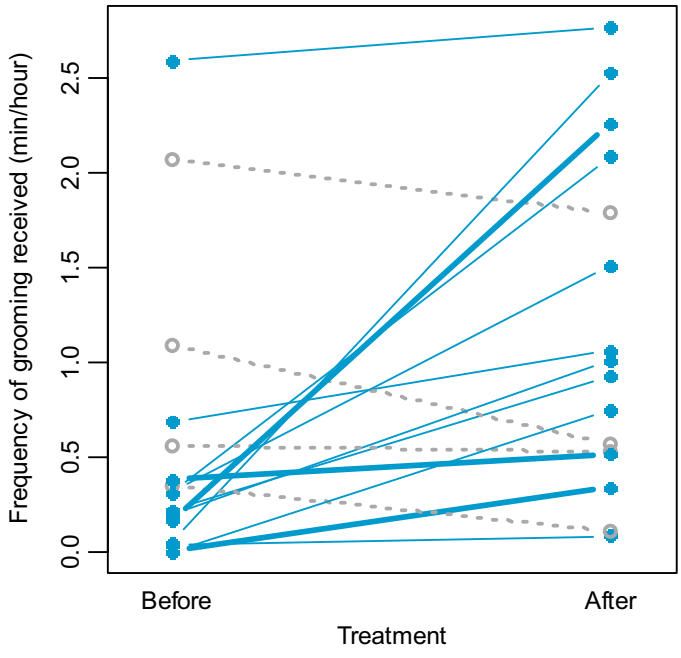

Fig. 1. Effect of antiparasitic treatment on the frequency of grooming received. Frequencies of grooming received were retrieved from a 6-week period extending from 3 weeks before treatment to 3 weeks after treatment. Each line represents one of the 16 deparasitized cases. Dotted gray lines with empty symbols represent individuals that received less grooming after than before treatment (n = 4). Solid blue lines with filled symbols represent individuals that received more grooming after than before treatment (n = 12). Of these 12 cases, the thick blue lines represent three individuals that received 10 times more grooming than represented in the figure (frequencies were divided by 10 to fit to the figure).

parasite infection. To the best of our knowledge, there are only two other reported cases of social avoidance strategies: Gregarious lobsters do not share dens with individuals infected by a lethal virus (*21*), and parasite-free bullfrog tadpoles do not swim with infected conspecifics (*22*). These strategies require efficient recognition mechanisms to discriminate parasitized conspecifics. Individual odors are likely cues of parasitism because infectious diseases often trigger changes in body odors (*23*). Moreover, mandrills often use olfaction to elaborate olfactory-guided behavioral responses (*24*), and they also often closely investigate conspecifics' perianal area. We therefore tested the hypothesis that the observed relationship between grooming behavior and protozoan status in mandrills is mediated by an olfactory mechanism.

We analyzed the chemical composition of 59 fecal samples collected from 30 individuals from the study population exhibiting different protozoan richness, using gas chromatography–mass spectrometry (GC-MS) analyses. Protozoan richness influenced the chemical composition of fecal odors (permutational multivariate analysis: $F = 0.10$, $R^2 = 0.11$, $P < 0.01$) (tables S4 and S5): the least chemically similar pairs of samples were those that showed the largest differences in protozoan richness. As for behavior, this relationship was particularly due to the presence of *B. coli* ($F = 13.3$, $R^2 = 0.07$, $P < 0.01$) in addition to that of *Entamoeba histolytica/dispar* ($F = 9.2$, $R^2 = 0.05$, $P = 0.01$) (Supplementary Materials) and *Entamoeba coli* ($F = 21.4$, $R^2 = 0.10$, $P < 0.001$) (table S4). Each of these parasites modified the chemical composition of fecal material: Among all possible pairs of fecal samples, the ones sharing a given parasite taxon are the most chemically similar pairs (Fig. 2).

Finally, we conducted 30 olfactory tests on 16 semi–free-ranging mandrills to experimentally confirm that olfactory cues contained in mandrills' fecal material provide information about host protozoan richness. During these tests, we presented pairs of fecal samples (rubbed on bamboo shoots) to temporarily isolated mandrills. Each pair of fecal samples, obtained not more than 3.5 months apart, was collected during the course of the study from the same individual from the free-ranging population. One sample contained at least two more protozoan taxa than the other sample. Individual subjects spent less time in proximity (<1 m) to the highly parasitized fecal sample than to the other, less parasitized fecal sample (Wilcoxon signed-rank test, $P = 0.014$; $n = 30$) (Fig. 3). This last result shows that olfaction is one possible proximate mechanism mediating the relationship between parasitism and sociality: Mandrills chemically discriminate fecal odors on the basis of the bearer's protozoan status.

This study reports social avoidance strategies combined with an accurate detection mechanism of contagious risk in a long-lived vertebrate. In the coevolutionary process linking parasites to their hosts, the latter continually evolve new strategies to counteract the costs of parasitism, whereas parasites adapt to bypass new host defense mechanisms (*25*). Although the evolution of the physiological immune system has been at the core of studies on host defense, theoretical studies have suggested that "behavioral immunity" could play a crucial role in host-parasite coevolutionary dynamics, driving the evolutionary trajectory of both species and influencing epidemiological parameters (*26*). The ability of mandrills to recognize and choose safe social partners, which we report here, helps explain how social species offset the costs of parasitism linked to frequent social interactions. However, social avoidance strategies should incur costs for the hosts because grooming is beneficial for both social partners involved (*18*). Therefore, hosts face a trade-off between these costs and the gain in fitness due to the avoidance of infection. These strategies are therefore expected to evolve only if the costs of being parasitized are high, that is, for virulent parasites. Interestingly, *B. coli*, which affects both grooming rates and fecal odorants in the studied mandrills, causes dysentery syndromes that are sometimes lethal in humans and other primates (*27*, *28*). In addition, *E. histolytica*, which is also detected in the population (Supplementary Materials), may cause

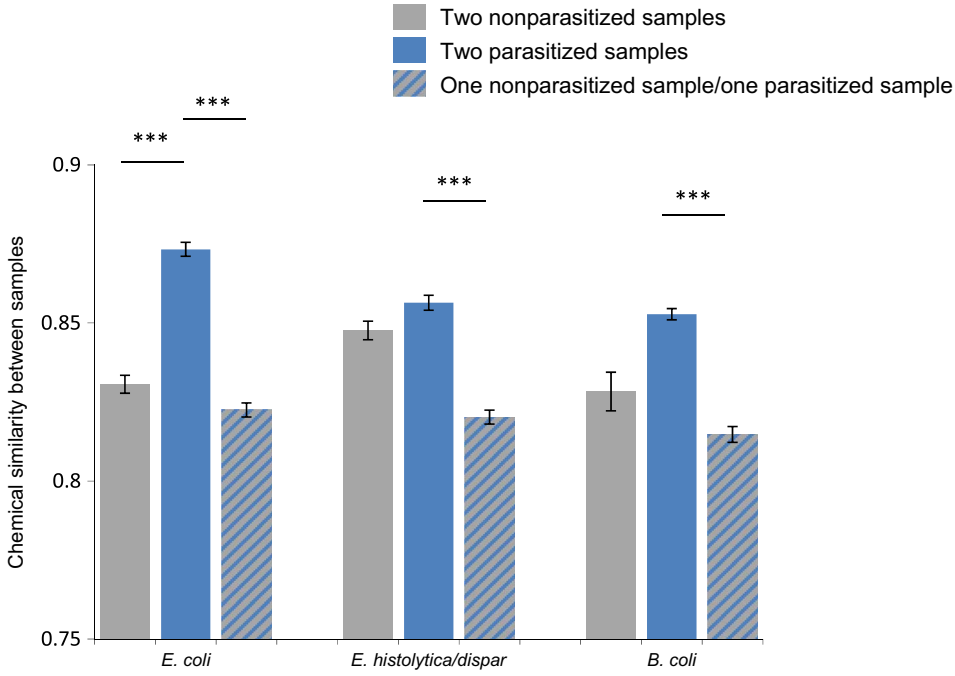

Fig. 2. Chemical similarity (mean ± SEM) between pairs of fecal samples with same or different protozoan statuses, for three protozoan taxa. Gray bars represent the average chemical similarity across pairs of nonparasitized fecal samples (*E. coli*, $n = 435$; *E. histolytica/dispar*, $n = 325$; and *B. coli*, $n = 105$), blue bars represent pairs of two parasitized fecal samples (*E. coli*, $n = 528$; *E. histolytica/dispar*, $n = 406$; and *B. coli*, $n = 946$), and hatched bars represent pairs of fecal sample with different protozoan statuses (*E. coli*, $n = 858$; *E. histolytica/dispar*, $n = 870$; and *B. coli*, $n = 660$). Significant differences are indicated for two-by-two comparisons (Kruskal-Wallis test, ***$P < 0.001$ for all instances).

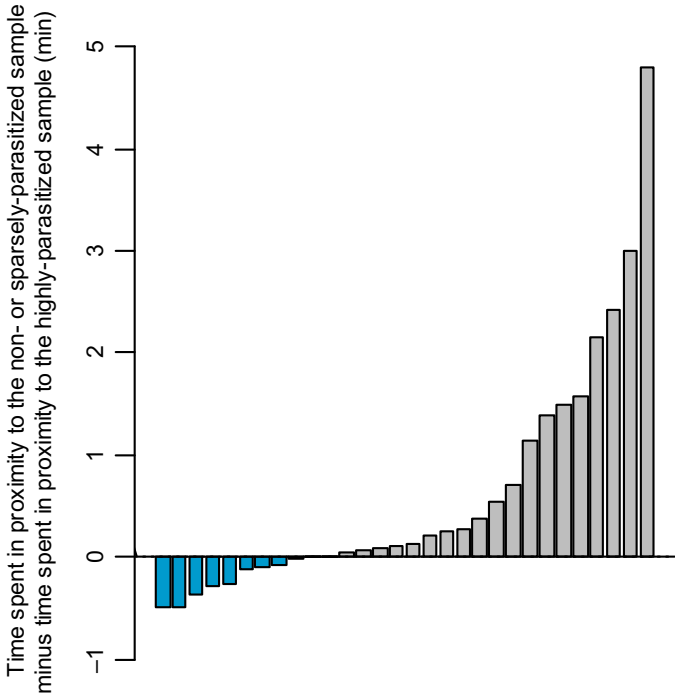

Fig. 3. Differences in time spent in proximity (<1 m) to non- or sparsely-parasitized samples versus highly-parasitized samples. n = 30 behavioral tests. Each bar represents the time difference (in minutes) recorded during one test. Blue bars, subjects spent more time in proximity to the highly-parasitized fecal sample than to the non- or sparsely-parasitized fecal sample (n = 9); gray bars, subjects spent more time in proximity to the non- or sparsely-parasitized fecal sample than to the highly parasitized fecal sample (n = 19). For two tests, subjects spent equal time near the two fecal samples.

fatal amoebiasis in humans and several other primates (*29*). Even low infection levels of gastrointestinal parasites may alter components of hosts' fitness (*30*, *31*) or lower performance and growth (*32*–*34*). Consequently, even if the protozoa studied here do not present conspicuous acute syndromes in mandrills, they are likely to act as selective agents driving the evolution of social avoidance strategies in hosts. Similarly, in human societies, the reported aversion to diseased individuals has been proposed to shape many aspects of our biology, some characteristics of human personality traits such as xenophobia and ethnocentrism, as well as cultural differences (*35*). Therefore, the constant coevolution between parasites and their hosts has not only driven the evolution of defense mechanisms but has also profoundly shaped the evolution of social systems.

MATERIALS AND METHODS

Study populations

We studied two populations of mandrills living in southern Gabon. The free-ranging population comprises ~120 to 130 habituated individuals living in a private park (Lékédi Park). This population was founded in 2002 when 36 mandrills were released from a captive population (*36*). Starting in 2003, wild males joined the group and reproduced with females born in captivity. In 2006, 29 other captive individuals were released into the initial group. During the first months after release, they were provisioned three to four times a week with bananas and monkey chow in quantities that never fulfilled their caloric need. The pace of this supplementation decreased rapidly (2008 to 2012) and completely ceased in April 2012. Tourists have regularly visited the group since 2004 (one visit a week on average), and the group consequently remained habituated to human presence. The frequency of tourist visitation decreased rapidly from 2012 to about one visit per month and ceased in June 2014. A long-term field study of this population—the "Mandrillus Project" [www.projetmandrillus.com (*37*)]—was set up in January 2012. At that time, more than 80% of the population was wild-born. Owing to daily monitoring, more than 100 individuals were individually known, and several field assistants collected day-long behavioral, demographic, and group-living data as well as fecal material from known animals. With the exception of the olfactory behavioral tests (see below), all analyses were performed on this population.

The second study population comprised ~200 individuals housed in two forested enclosures (6 and 3.5 ha; hereafter named "semi–free-ranging population") at the Centre International de Recherches Médicales de Franceville (CIRMF). These animals forage freely, but animal keepers supplemented their diet with fruits, vegetables, and monkey chow on a daily basis. The food is placed in small closed areas that allow isolating animals for particular protocols such as behavioral tests. Tests based on olfactory cues were performed on individuals from this population.

Do parasitized individuals harbor protozoan cysts on external body parts?

Experimental protocol.

Taking advantage of a scheduled trapping session, in July 2014, we estimated the protozoan richness (total number of protozoan taxa) (*17*) of 36 anesthetized individuals by taking skin smears from three different parts of their body. We also collected their feces. The anesthetics used were IMALGENE 1000 (7 mg/kg for adults and 5 mg/kg for juveniles) and xylazine (3 mg/kg for adults and 5 mg/kg for juveniles). The skin smears for each individual were obtained by rubbing a gauze on the perianal area and another one on other parts of the body (chest, arm, leg, and back). We carried out qualitative parasitological analyses on the three samples (two smears and fecal material) of each of the 36 mandrills. Coprological analyses were performed using sedimentation, following standard procedures (*17*). We used a similar method to analyze the skin smears by just replacing the fecal material with the gauze. We recorded protozoan cysts according to their morphological characteristics (*17*). Of the seven protozoan taxa known to infect individuals from the studied free-ranging population (*17*), six were found in the fecal material of the 36 studied individuals. Of these six protozoan taxa, *Coccidia sp.* parasitized only five individuals and was therefore not included in the statistical analyses discussed below.

Statistical analyses.

For each of the five studied protozoan taxa, we first performed Fisher's test to compare the proportions of positive skin smears in parasitized versus nonparasitized individuals. Even nonparasitized animals may still harbor protozoan cysts on their fur because of, for example, contact with soiled environments and/or a nondetectable low level of parasitism. For the parasitized individuals, we performed additional Fisher's tests to compare the proportions of positive skin smears when collected from the perianal area versus from other body parts. We adjusted P values for multiple comparisons using Bonferroni corrections. All statistical analyses, here and below, were performed using R version 3.0.3.

How does protozoan status influence behavioral patterns of allogrooming and spatial associations?

Behavioral observations.

From October 2012 to April 2015, trained observers performed behavioral observations on known animals from the free-ranging population, using a 5-min focal sampling (*38*). The observers had no knowledge

of the degree of protozoan infection of any of the mandrills. In the following analyses, we kept only individuals for whom at least 30 min of focal time was available in a given month (mean ± SEM, 175 ± 17 min of focal time including 3.4 ± 0.5 min of grooming received and 5.5 ± 1.8 min of grooming given per individual per month).

We calculated a monthly frequency of grooming received and given by dividing the time spent being groomed by, or grooming, any conspecific by the total focal time performed on this animal this month. To control for variations under ecological conditions and differences in sampling effort across months, we calculated monthly indices of grooming received and given by dividing the previous frequencies by the frequencies of grooming received and given (respectively) recorded in the whole population [following the study of Silk *et al.* (*18*)]. High monthly indices of grooming received or given (>1) represented individuals that received, or gave, more grooming than the average individual found in the population during that month. Starting from July 2014, we used ad libitum data in addition to the focal data to further examine whether each allogrooming event (considered here as a discrete variable) included or not the perianal area (yes or no).

Protozoan status.

We have monitored the presence of parasites in the feces of known individuals since 2012 (*17*). We assessed monthly individual protozoan richness by recording the average number of protozoan taxa in all fecal samples obtained for each individual each month. We restricted our analyses to those months for which at least three fecal samples were available per studied animal (mean ± SEM, 4.5 ± 0.17 fecal samples per individual per month). We further assessed monthly individual specific protozoan status corresponding to the average number of positive samples—for each of the seven protozoan taxa known to occur in the mandrill population—in all samples collected from one individual during that month. In preliminary analyses, we checked that the origin of the studied animals (captive-born versus wild-born) did not influence protozoan richness and specific status (*t* tests, $P > 0.1$ in all comparisons).

Spatial association.

In July 2014, we equipped a subset of the population with proximity data loggers (ELA Innovation). These small radio transceiver units record spatial proximity among equipped individuals, that is, from a few centimeters (<10 cm) to 2 to 3 m, through the periodical exchange of radio packets. The proximity logger system consists of an ultrahigh frequency transceiver that broadcasts a unique ID code, while simultaneously "listening" to others. Each unit also contains a very high frequency transmitter, which pulses at a periodic rate like a beacon. At intervals of 7 min (from 6:00 a.m. to 6:00 p.m.) or 1 hour (at night) and during a 20-s period, the receiving unit queries an onboard real-time clock and begins counting when one or more units are within the range and their detected ID code(s). The ID code, date, and time of the encounter are stored in a nonvolatile memory in the hardware of the collar devices and are downloaded daily by field observers using a portable reading device. We estimated two indices of spatial associations (that is, degrees and contact). "Degrees" measure the number of individuals that have been in contact with the focal individual on a given day, whereas "contact" measures all contacts with the focal individual (regardless of the identities of the contacting animals) on a given day.

Statistical analyses.

To study the relationship between allogrooming behavior and protozoan status, we performed LMM (nlme package, R). We investigated the effect of monthly protozoan richness as well as monthly specific protozoan status on indices of grooming received and given. These models were run using individuals that were observed (i) for at least 30 min and (ii) from whom at least three fecal samples were collected in that month (25 individuals: 13 females and 12 males aged 1.5 to 22 years, representing $n = 105$ individual.month). We log-transformed indices of grooming to meet normality assumptions. We used a multimodel approach to test for all possible combinations of predictors that best explained the variables of interest [that is, models associated with the highest Akaike weights based on corrected AIC (AICc)] (*20*). We then used model averaging with the R package MuMIn to calculate the average values of coefficients of the predictors included in the subset of models with a ΔAICc inferior to 10. We considered the following as predictors: the individual's age (continuous predictor, in years), sex (categorical predictor, two modalities), and dominance rank (categorical predictor, three modalities) of the studied animals as well as their monthly protozoan richness or their specific protozoan status. Dates of birth of released individuals born in captivity were exactly known owing to the daily monitoring at CIRMF. The exact age of most individuals from the free-ranging population was known for those born after the beginning of the Mandrillus Project. The age of older animals born in the wild was estimated using general body condition and, for some individuals, patterns of tooth eruption and wear. Female and male monthly dominance ranks were evaluated using the outcomes of approach-avoidance interactions. We attributed their mother's rank to the four studied males below 5 years of age. For both sexes, we considered three classes of dominance rank (low, middle, and high). "Dominance" was only included in the model in interaction with "sex" because dominance is set differently according to sex (*17*). We further included a random effect linked to the individual's identity and added a structured temporal autocorrelation of the form ARMA (autoregressive moving average), to correct for potential biases due to autotemporal correlations within the data set.

Second, to study the relationship between spatial association (that is, degrees and contact) and protozoan status, we used a similar multimodel LMM approach. We log-transformed the response variable contact to meet normality assumptions. We considered the sex, age, dominance rank (in interaction with sex), and monthly protozoan richness as fixed predictors and the individual's identity as a random effect. We associated to daily degrees or contact the corresponding monthly protozoan richness for individuals equipped with proximity data loggers from whom at least three fecal samples were collected in that month (13 individuals: 11 females and 2 males aged 6 to 16 years, representing $n = 40$ individual.month and $n = 1026$ individual.day). As in the analyses described in the previous paragraph, we added a structured autocorrelation of the form ARMA.

Finally, to study the relationship between monthly protozoan status of the groomee and the probability of receiving grooming events that included the perianal area, we performed GLMM, using a multimodel approach. We used a binomial distribution with a logit-link function, by treating grooming events as "yes" (those including the perianal area) and "no" (those that did not include the perianal area). We used the grooming data recorded from behavioral focal samplings and ad libitum behavioral observations. We further considered age, sex, and dominance rank in interaction with sex as fixed predictors and the individual's identity as a random effect. As in the two previous analyses, we included only individuals from whom at least three fecal samples were collected in a given month (14 individuals: eight females and six males aged 4 to 22 years, representing $n = 31$ individual.month).

How does experimental antiparasitic treatment influence allogrooming behavior?

Antiparasitic treatment.

From January 2014 to August 2015, we deparasitized 15 adult individuals on 16 occasions (one individual was deparasitized twice) using

metronidazole administered either orally (n = 3) or by intravenal perfusion (n = 13). During regular behavioral monitoring, individuals were deparasitized by oral ingestion of small pieces of bananas containing 400 mg of metronidazole for five consecutive days or 5 days within a 1-week window. During trapping sessions (July to November 2014 and May 2015), individuals received slow (30 to 40 min) perfusion of metronidazole (5 mg/ml) (adult males, 200 ml; adult females, 100 ml). We were unable to systematically collect fecal samples after treatment. However, the medical drug we used is highly recommended to eradicate protozoa in veterinary and human medicines. We therefore assumed that the intensity of protozoan infection decreased in treated animals compared to untreated individuals. As controls, we used nine individuals that were captured but not deparasitized. For treated and control individuals, we calculated frequencies of allogrooming received and given by dividing the time spent by the animals receiving or giving grooming behavior by the total time the individual was observed during the two periods: 3 weeks before treatment and 3 weeks after treatment (for the 3 weeks before treatment: mean ± SEM, 204 ± 39 min of focal time per individual including 3.5 ± 0.8 min of grooming received and 8.7 ± 3.8 min of grooming given; for the 3 weeks following treatment: mean ± SEM, 225 ± 41 min of focal time per individual including 10.1 ± 1.9 min of grooming received and 11.8 ± 4.8 min of grooming given). These 3-week windows were long enough to allow the acquisition of good estimates of grooming indices. A longer period of observation would have greatly increased the probability of reinfection in treated individuals.

Statistical analyses.

We performed Wilcoxon signed-rank tests to compare the frequencies of grooming received and given 3 weeks before and 3 weeks after treatment or after anesthesia without treatment for control individuals. We performed two additional Wilcoxon signed-rank tests by alternatively considering only one deparasitizing event for the individual that was treated twice. This modification did not change the results.

Exploration of an olfactory-guided mechanism to parasite avoidance

Chemical analyses.

Odor sampling, extraction and GC-MS analyses. We collected odors from 59 fecal samples obtained from 30 individuals from the free-ranging population (20 females aged 4 to 19 years and 10 males aged 4 to 14 years) with different protozoan statuses. A few hours after collection, we stored a small pellet of fecal material, previously mixed for homogenization, in a solvent-washed chromatography vial for 1 to 3 months at −20°C before shipping from Gabon to France. In France, samples were stored at −80°C until analyses were conducted. We used the solid-phase microextraction method to extract volatile compounds emitted by fecal material. After thawing the samples for 20 min, we introduced an adsorbent-coated fiber into the collection vial. Chemical extraction was run for 20 min. We collected and extracted "blanks" using similar protocols. Blanks included a random collection of volatiles present in the area where fecal samples were collected and in the laboratory where analyses were performed. We analyzed the 59 extracts on a GC-MS (Shimadzu GCMS-QP2010 Plus), equipped with a generalist ZB-5MSi (Phenomenex) capillary column (L = 30.0 m; thickness, 0.25 µm; Ø = 0.25 mm). Samples were injected in splitless mode. We set the injector temperature at 200°C and the ion source at 250°C and used helium as the carrier gas (constant linear velocity, 35 cm/s). Masses were scanned from 50 to 525 uma (unified mass in electron ionization mode in 1 s). We used the following method to discriminate chemical compounds: total run time lasted 43 min by ramping the temperature from 40° to 200°C at a rate of 5°C/min, then from 200° to 240°C at a rate of 16°C/min (held at 240°C for 4 min). We manually detected volatile compounds by integrating peaks that comprised at least 0.05% of the overall area of the chromatogram and that were not present in blank chromatograms.

Because of the complex chemical composition of fecal odors, we used a semiautomatic method to group volatile compounds with similar mass spectra into clusters, without prior alignment of retention time, based on the recognition of similar mass spectra rather than on retention times or indices (*39*). We manually checked retention times to control the quality of the clusters. We subsequently identified the corresponding molecule for each cluster by consulting the National Institute of Standards and Technology (v.05) and Wiley (9th edition) libraries, comparing the mass spectra of representative peaks of each cluster with the reference database. Finally, we created a matrix of presence/absence (0/1) for each of the major compounds identified present in at least 20% of the chromatograms analyzed (n = 75 molecules) (table S5) to simplify a highly complex database composed of more than 150 molecules and to limit detection or identification errors. We generated a chemical distance matrix for all possible pairs of fecal samples. We used a probabilistic index (Raup-Crick) for presence/absence data that considers samples sharing rare volatile compounds as more similar than samples sharing common volatile compounds.

Statistical analyses. We first studied whether differences in protozoan richness between all possible pairs of fecal samples influenced their chemical similarities, using permutation analyses (5000) with pseudo–F ratios (vegan package, adonis procedure). We controlled for the following predictors: the time lag between fecal sample collection and GC-MS analyses (quantitative predictor), the season (categorical predictor, four modalities: long-humid, short-humid, long-dry, and short-dry seasons) (*17*), and the sex of individuals sampled (categorical predictor, two modalities). We added an individual stratum to this model to control for multiple sampling on the same animals. In additional models, we tested for any relationship between chemical similarity and the presence (or not) of each protozoan taxon, except *Pseudolimax butschlii*, which was not found in the samples analyzed, controlling for the same predictors as above and adjusting P values for multiple comparisons using Bonferroni corrections. For the three protozoan taxa found to modify the chemical composition of fecal samples, we performed Kruskal-Wallis tests to compare the chemical similarity between pairs of fecal samples with identical or different protozoan statuses (Fig. 2), adjusting P values for multiple comparisons using Bonferroni corrections.

Olfactory tests.

Fecal sample collection. Fecal samples were collected from 11 individuals (seven females and four males aged 4 to 14 years) from the free-ranging population that served as odor donors to constitute a total of 15 pairs of fecal samples. Each pair of fecal samples used in each behavioral test was collected from the same individual not more than 3.5 months apart. We selected pairs of fecal samples that differed the most in their protozoan richness: one fecal sample ("the highly-parasitized" one) was parasitized by at least three different protozoan taxa, whereas the other ("the non- or sparsely-parasitized" one) was parasitized by zero to two protozoan taxa. There was a difference in richness of at least two protozoan taxa between paired samples. We avoided potential biases due to storage time by randomizing the age of the fecal sample and the associated protozoan status (the oldest sample of each pair was the most parasitized in 7 of 15 pairs and the least parasitized in 8 of 15 pairs). Finally, to avoid an obvious potential source of biases, we never used fecal samples collected from sexually receptive females.

Experimental design. In September 2014, we performed 30 behavioral tests on 16 semi–free-ranging mandrills ("subjects") aged 4 to 26 years in their living enclosure [one to three session(s) of 10 min per individual] and temporarily isolated from their companions. Before each session, subjects entered into a first closed area (C1) that communicates with a similar area (C2) in which behavioral tests were performed. In C2, we attached three bamboo shoots (30 cm long) in a line along the fence, each 2 m away from the preceding one and facing the observers. First, during preliminary experiments, we rubbed an herbaceous plant on the three bamboo shoots to verify that individuals were not more attracted toward the bamboo located on the middle or on the edges ($n = 6$ tests performed). During the actual experimental stage, we rubbed a pellet of fecal samples (one sample per bamboo shoot) thawed 20 min before use at the middle of the two external bamboos ("scented bamboos"). We alternated the position of the highly-parasitized and non- or sparsely-parasitized fecal samples between each test. As a control, we rubbed an herbaceous plant on the central, control bamboo shoot. Subjects were in visual contact with the observer during deposition of fecal material on the bamboo shoots.

Each session started when the subject entered into C2. An observer, ignorant of the protozoan richness of the fecal samples, faced the three bamboos and recorded the number of olfactory investigations performed (sniffing, touching, or licking the bamboo) as well as the time spent in proximity (<1 m) to each bamboo. At the end of the test, the individual was released into C1. After each test, the enclosure was cleaned, and another pair of fecal samples was rubbed onto new bamboo shoots. Fecal samples were stored in an icebox between tests. To avoid multiple freeze-thawing, we only used each pair of fecal samples one to three times in the same half-day, depending on the number of tested individuals.

Statistical analyses. In a first preliminary analysis, we confirmed that focal individuals were not more attracted toward one localization using Wilcoxon signed-rank tests to compare the number of olfactory investigations performed toward and the time spent in proximity to each of the three bamboo shoots ($P > 0.1$ for all two-by-two comparisons). In a second preliminary analysis, we verified that mandrills behaved differently toward the two outer, scented bamboo shoots compared to the central, control one. We used Wilcoxon signed-rank tests to compare the number of olfactory investigations performed toward and the time spent in proximity to the control bamboo to the average number of olfactory investigations toward and the average time spent in proximity to the two scented bamboos. We found that subjects, on average, investigated more and spent more time near the scented bamboos than the control bamboo ($P < 0.01$ for both variables). By investigating a conspecific's fecal material, mandrills may obtain crucial information on the bearer's social, sexual, and, whenever possible, parasite status, which may explain why fecal samples induced more interest from subjects than the control, unscented bamboo. Thereafter, we performed Wilcoxon signed-rank tests to compare the number of olfactory investigations performed toward and the time spent in proximity to each of the two scented bamboos.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/4/e1601721/DC1

Supplementary Results

table S1. Results of coprological analyses performed on skin smears, according to the individual's protozoan status.

table S2. Effects of each protozoan taxon on the index of grooming received.

table S3. Effects of different predictors on (A) daily degree and (B) daily number of contacts.

table S4. Effects of different predictors on the chemical similarity between pairs of fecal samples.

table S5. Major volatile compounds ($n = 75$) found in the analyzed fecal samples and their chemical family.

fig. S1. Effect of the capture (without medical treatment) on the frequency of grooming received.

REFERENCES AND NOTES

1. P. M. Kappeler, S. Cremer, C. L. Nunn, Sociality and health: Impacts of sociality on disease susceptibility and transmission in animal and human societies. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140116 (2015).
2. S. Altizer, C. L. Nunn, P. H. Thrall, J. L. Gittleman, J. Antonovics, A. A. Cunningham, A. P. Dobson, V. Ezenwa, K. E. Jones, A. B. Pedersen, M. Poss, J. R. C. Pulliam, Social organization and parasite risk in mammals: Integrating theory and empirical studies. *Annu. Rev. Ecol. Evol. Syst.* **34**, 517–547 (2003).
3. J. L. Rifkin, C. L. Nunn, L. Z. Garamszegi, Do animals living in larger groups experience greater parasitism? A meta-analysis. *Am. Nat.* **180**, 70–82 (2012).
4. C. L. Nunn, J. L. Gittleman, J. Antonovics, Promiscuity and the primate immune system. *Science* **290**, 1168–1170 (2000).
5. B. L. Hart, Behavioural defences in animals against pathogens and parasites: Parallels with the pillars of medicine in humans. *Philos. Trans. R. Soc. B Biol. Sci.* **366**, 3406–3417 (2011).
6. V. O. Ezenwa, Selective defecation and selective foraging: Antiparasite behavior in wild ungulates? *Ethology* **110**, 851–862 (2004).
7. C. Sarabian, A. J. J. MacIntosh, Hygienic tendencies correlate with low geohelminth infection in free-ranging macaques. *Biol. Lett.* **11**, 20150757 (2015).
8. M. Chapuisat, A. Oppliger, P. Magliano, P. Christe, Wood ants use resin to protect themselves against pathogens. *Proc. Biol. Sci.* **274**, 2013–2017 (2007).
9. B. L. Hart, Behavioral adaptations to pathogens and parasites: Five strategies. *Neurosci. Biobehav. Rev.* **14**, 273–294 (1990).
10. V. A. Curtis, Infection-avoidance behaviour in humans and other animals. *Trends Immunol.* **35**, 457–464 (2014).
11. W. J. Freeland, Pathogens and the evolution of primate sociality. *Biotropica* **8**, 12–24 (1976).
12. S. A. Budischak, A. E. Jolles, V. O. Ezenwa, Direct and indirect costs of co-infection in the wild: Linking gastrointestinal parasite communities, host hematology, and immune function. *Int. J. Parasitol. Parasites Wildl.* **1**, 2–12 (2012).
13. D. M. Tompkins, A. M. Dunn, M. J. Smith, S. Telfer, Wildlife diseases: From individuals to ecosystems. *J. Anim. Ecol.* **80**, 19–38 (2011).
14. O. Berger-Tal, S. Bar-David, Recursive movement patterns: Review and synthesis across species. *Ecosphere* **6**, 1–12 (2015).
15. C. E. J. Kennedy, J. A. Endler, S. L. Poynton, H. McMinn, Parasite load predicts mate choice in guppies. *Behav. Ecol. Sociobiol.* **21**, 291–295 (1987).
16. N. Bos, T. Lefèvre, A. B. Jensen, P. D'Ettorre, Sick ants become unsociable. *J. Evol. Biol.* **25**, 342–351 (2012).
17. C. Poirotte, D. Basset, E. Willaume, F. Makaba, P. M. Kappeler, M. J. E. Charpentier, Environmental and individual determinants of parasite richness across seasons in a free-ranging population of mandrills (*Mandrillus sphinx*). *Am. J. Phys. Anthropol.* **159**, 442–456 (2016).
18. J. B. Silk, S. C. Alberts, J. Altmann, Social bonds of female baboons enhance infant survival. *Science* **302**, 1231–1234 (2003).
19. M. S. Mooring, D. T. Blumstein, C. J. Stoner, The evolution of parasite-defence grooming in ungulates. *Biol. J. Linn. Soc.* **81**, 17–37 (2004).
20. K. P. Burnham, D. R. Anderson, *Model Selection and Multimodel Inference: A Practical Information-Theoric Approach* (Springer-Verlag, 2002).
21. D. C. Behringer, M. J. Butler, J. D. Shields, Avoidance of disease by social lobsters. *Nature* **441**, 421 (2006).
22. J. M. Kiesecker, D. K. Skelly, K. H. Beard, E. Preisser, Behavioral reduction of infection risk. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9165–9168 (1999).
23. F. Prugnolle, T. Lefèvre, F. Renaud, A. P. Møller, D. Missé, F. Thomas, Infection and body odours: Evolutionary and medical perspectives. *Infect. Genet. Evol.* **9**, 1006–1009 (2009).
24. M. J. E. Charpentier, S. Mboumba, C. Ditsoga, C. M. Drea, Nasopalatine ducts and flehmen behavior in the mandrill: Reevaluating olfactory communication in Old World primates. *Am. J. Primatol.* **75**, 703–714 (2013).
25. L. Van Valen, A new evolutionary law. *Evol. Theory* **1**, 1–30 (1973).
26. M. H. Bonds, D. D. Keenan, A. J. Leidner, P. Rohani, Higher disease prevalence can induce greater sociality: A game theoretic coevolutionary model. *Evolution* **59**, 1859–1866 (2005).
27. A.-P. Bellanger, E. Scherer, A. Cazorla, F. Grenouillet, Dysenteric syndrome due to *Balantidium coli*: A case report. *New Microbiol.* **36**, 203–205 (2013).
28. T. Ferry, D. Bouhour, F. De Monbrison, F. Laurent, H. Dumouchel-Champagne, S. Picot, M. A. Piens, P. Granier, Severe peritonitis due to *Balantidium coli* acquired in France. *Eur. J. Clin. Microbiol.* **23**, 393–395 (2004).

29. R. Ulrich, M. Böer, V. Herder, I. Spitzbarth, M. Hewicker-Trautwein, W. Baumgärtner, P. Wohlsein, Epizootic fatal amebiasis in an outdoor group of Old World monkeys. *J. Med. Primatol.* **39**, 160–165 (2010).
30. M. Asghar, D. Hasselquist, B. Hansson, P. Zehtindjiev, H. Westerdahl, S. Bensch, Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* **347**, 436–438 (2015).
31. A. Stien, R. J. Irvine, E. Ropstad, O. Halvorsen, R. Langvatn, S. D. Albon, The impact of gastrointestinal nematodes on wild reindeer: Experimental and cross-sectional studies. *J. Anim. Ecol.* **71**, 937–945 (2002).
32. A. R. Sykes, R. L. Coop, Intake and utilization of food by growing sheep with abomasal damage caused by daily dosing with *O. circuminata* larvae. *J. Agric. Sci.* **88**, 671–677 (1977).
33. V. Alzaga, J. Vicente, D. Villanua, P. Acevedo, F. Casas, C. Gortazar, Body condition and parasite intensity correlates with escape capacity in Iberian hares (*Lepus granatensis*). *Behav. Ecol. Sociobiol.* **62**, 769–775 (2008).
34. C. A. Bradley, S. Altizer, Parasites hinder monarch butterfly flight: Implication for disease spread in migratory hosts. *Ecol. Lett.* **8**, 290–300 (2005).
35. M. Schaller, D. R. Murray, A. Bangerter, Implications of the behavioural immune system for social behaviour and human health in the modern world. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140105 (2015).
36. P. Peignot, M. J. E. Charpentier, N. Bout, O. Bourry, U. Massima, O. Dosimont, R. Terramorsi, E. J. Wickings, Learning from the first release project of captive-bred mandrills *Mandrillus sphinx* in Gabon. *Oryx* **42**, 122–131 (2008).
37. T. Brockmeyer, P. M. Kappeler, E. Willaume, L. Benoit, S. Mboumba, M. J. E. Charpentier, Social organization and space use of a wild mandrill (*Mandrillus sphinx*) group. *Am. J. Primatol.* **77**, 1036–1048 (2015).
38. J. Altmann, Observational study of behavior: Sampling methods. *Behaviour* **49**, 227–266 (1974).
39. F. Nicolè, Y. Guitton, E. A. Courtois, S. Moja, L. Legendre, M. Hossaert-McKey, MSeasy: Unsupervised and untargeted GC-MS data processing. *Bioinformatics* **28**, 2278–2280 (2012).

Acknowledgments: We are grateful to past and present field assistants of the Mandrillus Project who collected daily behavioral data on the free-ranging population and to A. Meyer, L. Gatel, and S. Valentin for their assistance during trapping sessions. We thank the personnel at CIRMF for their assistance in behavioral tests, particularly C. Moussadji. Chemical analyses were performed at the Plateforme d'Analyses Chimiques en Ecologie, technical facilities of the Centre Méditerranéen Environnement et Biodiversité (CeMEB, Montpellier, France), and we thank N. Barthes for the assistance. We also thank D. McKey and L. Dormont for their helpful advice. **Funding:** This study was funded by a grant from the Deutsche Forschungsgemeinschaft (DFG; KA 1082-20-1) to M.J.E.C. and P.M.K., the Station d'Etudes en Ecologie Globale (SEEG, INEE-CNRS) to M.J.E.C., the Laboratoire International Associé (LIA, CIRMF and INEE-CNRS) to M.J.E.C., and the Projets Exploratoires Premier Soutien ECOSAN (PEPS, INEE-CNRS) to M.J.E.C. **Author contributions:** C.P. and M.J.E.C. designed the study. C.P., M.J.E.C., and F.M. analyzed the data, and with P.M.K. wrote the article. C.P. and A.H. performed behavioral tests. C.P. and P.M.B. collected behavioral data on the wild population. M.J.E.C. and E.W. contributed to long-term data collection. M.J.E.C. managed the long-term databases used for this article. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** This study was authorized by the CENAREST Institute (permit no. AR0024/16/MESRS/CENAREST/CG/CST/CSAR). This is Project Mandrillus publication no. 7. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from M.J.E.C. (marie.charpentier@cefe.cnrs.fr).

Submitted 25 July 2016
Accepted 10 February 2017
Published 7 April 2017
10.1126/sciadv.1601721

Citation: C. Poirotte, F. Massol, A. Herbert, E. Willaume, P. M. Bomo, P. M. Kappeler, M. J. E. Charpentier, Mandrills use olfaction to socially avoid parasitized conspecifics. *Sci. Adv.* **3**, e1601721 (2017).

ScienceAdvances
AAAS

Mandrills use olfaction to socially avoid parasitized conspecifics

Clémence Poirotte, François Massol, Anaïs Herbert, Eric Willaume, Pacelle M. Bomo, Peter M. Kappeler and Marie J. E. Charpentier (April 7, 2017)
Sci Adv 2017, 3:.
doi: 10.1126/sciadv.1601721