Genetic diversity and reproductive success in mandrills (*Mandrillus sphinx*)

M. Charpentier*†‡§, J. M. Setchell†‡¶, F. Prugnolle‖, L. A. Knapp¶, E. J. Wickings†, P. Peignot†, and M. Hossaert-McKey*

*Centre d'Ecologie Fonctionnelle et Evolutive, Unite Mixte de Recherche 5175, Centre National de la Recherche Scientifique, 1919 Route de Mende, 34293 Montpellier Cedex 5, France; †Unité de Génétique des Ecosystèmes Tropicaux, Centre International de Recherches Médicales de Franceville, BP 769, Franceville, Gabon; ¶Department of Biological Anthropology, University of Cambridge, Downing Street, Cambridge CB2 3DZ, United Kingdom; and ‖Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614

Edited by Tomoko Ohta, National Institute of Genetics, Mishima, Japan, and approved September 29, 2005 (received for review August 25, 2005)

Recent studies of wild animal populations have shown that estimators of neutral genetic diversity, such as mean heterozygosity, are often correlated with various fitness traits, such as survival, disease susceptibility, or reproductive success. We used two estimators of genetic diversity to explore the relationship between heterozygosity and reproductive success in male and female mandrills (*Mandrillus sphinx*) living in a semifree ranging setting in Gabon. Because social rank is known to influence reproductive success in both sexes, we also examined the correlation between genetic diversity and social rank in females, and acquisition of alpha status in males, as well as length of alpha male tenure. We found that heterozygous individuals showed greater reproductive success, with both females and males producing more offspring. However, heterozygosity influenced reproductive success only in dominant males, not in subordinates. Neither the acquisition of alpha status in males, nor social rank in females, was significantly correlated with heterozygosity, although more heterozygous alpha males showed longer tenure than homozygous ones. We also tested whether the benefits of greater genetic diversity were due mainly to a genome-wide effect of inbreeding depression or to heterosis at one or a few loci. Multilocus effects best explained the correlation between heterozygosity and reproductive success and tenure, indicating the occurrence of inbreeding depression in this mandrill colony.

correlation heterozygosity-fitness | genome-wide inbreeding | primate | social rank | reproduction

Offspring born to closely related parents often show reduced fitness, a phenomenon known as inbreeding depression (1); this is typically due to increased homozygosity at loci affecting fitness, either by permitting the expression of recessive deleterious alleles or by reducing heterozygote advantage (2). Inbreeding avoidance is thought to underlie the evolution of sex-biased dispersal (3, 4) and has important implications for conservation (5).

Most studies of the relationship between inbreeding and fitness have been carried out using domestic or captive animals (reviewed in refs. 6–8). Studies of the influence of inbreeding on overall fitness in natural, or seminatural, populations remain relatively rare because accumulating the generations of pedigree information necessary to calculate inbreeding coefficients requires long-term study, particularly for long-lived organisms. Moreover, studies using inbreeding coefficients are limited where mating between close relatives is infrequent in a population (9). An alternative approach to the use of inbreeding coefficients is to exploit the fact that inbreeding reduces heterozygosity (10).

The development of new genetic techniques (e.g., microsatellite analysis), and of estimators of genetic diversity which improve on simple single-locus measures of heterozygosity, has led to increasing numbers of studies of inbreeding depression in wild populations over the past 10 years. For example, Coulson *et al.* (11) developed mean d^2 as an estimator of the evolutionary similarity of alleles. Although more recent studies suggest that mean d^2 is appropriate only under rather unusual circumstances (12, 13), two further estimates of heterozygosity, standardized heterozygosity (14) and internal relatedness (15), show high correlations across a range of species (15, 16). The former allows incomplete genotyping, whereas the latter weighs allele sharing by the frequencies of the alleles in the population; thus, both are theoretically more informative than simple observed heterozygosity.

A growing number of studies have shown that these estimators of genetic diversity are correlated with a range of fitness components, including survival, disease susceptibility, and reproductive success (11, 14–25). The general consensus of these studies is that an association exists between multilocus heterozygosity and components of fitness (26). In most cases, inbreeding depression is proffered as the likely underlying mechanism. However, recent theoretical and empirical studies have shown that, when variation in inbreeding is low in a population, multilocus (i.e., microsatellite) heterozygosity is only weakly correlated with inbreeding coefficients obtained from pedigree data (27, 28), even when the data set comprises several hundred microsatellite markers (27).

These studies raise vexing questions concerning inbreeding depression. Most importantly, if microsatellite heterozygosity is such a poor estimator of genome-wide inbreeding, why do so many studies report correlations between heterozygosity and inbreeding, and ultimately fitness? This may be because the study populations show sufficient variation in inbreeding to allow heterozygosity to be a good estimate of inbreeding. Alternatively, Balloux *et al.* (27) have proposed various explanations for this phenomenon, including publishing bias (29). Otherwise, correlations between heterozygosity and inbreeding may be due to nonrandom choice of neutral markers which are generally selected due to their genetic variability, and may be located in genomic regions under balancing selection. Balloux *et al.* (27) also suggested that genotypic disequilibrium may generate single locus associations, where a small number of strongly overdominant genes generate spurious associations with multiple neutral loci, and proposed that future studies should seek heterozygosity-fitness associations that appear marker specific (e.g., refs. 16 and 30).

Long-term studies of isolated populations can provide valuable data concerning inbreeding, heterozygosity, and fitness (31). Here we examine the relationship between genetic diversity and reproductive success in a semifree ranging population of mandrills (*Mandrillus sphinx*, Cercopithecinae), at the Centre International de Recherches Médicales de Franceville (CIRMF), Gabon. The CIRMF mandrill colony was established in 1983, with 15 founder animals originating from the wild (32); no animals have been introduced subsequently. Animals cannot emigrate and reproduce in their natal groups. However, it would be impossible to address our research question in the wild, and mandrills are an interesting

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: CIRMF, Centre International de Recherches Médicales de Franceville; HO, observed heterozygosity per individual; IR, internal relatedness.

‡M.C. and J.M.S. contributed equally to this work.

§To whom correspondence should be addressed. E-mail: mariecharp@yahoo.fr.

model species for studies of inbreeding because of a lack of studies in primates and high reproductive skew among males (alpha males in the colony sire 76% of offspring; ref. 33). Inbreeding avoidance appears to occur in the colony, particularly between first-order relatives (33).

We estimated genetic diversity using heterozygosity and internal relatedness. Although precise estimation of inbreeding coefficients from genetic data usually requires a large number of markers, particular situations, including highly skewed mating systems such as the polygynous reproductive system studied here, allow valid estimation of inbreeding despite the use of only a small number of loci (27). We estimated reproductive success as the number of offspring sired by potentially reproductive males and the number of surviving offspring produced by females. Because social rank is known to significantly influence reproductive success in both sexes (34), we also examined the relationship between genetic diversity and dominance rank. Finally, we examined whether the relationships observed between genetic diversity and reproductive success were due to genome-wide heterozygosity at unlinked genes, or to one or more neutral loci being linked to other loci under selection (27).

Methods

Study Species and Population. Mandrills are primarily terrestrial primates, living in multimale, multifemale groups, found in the tropical forests of western Central Africa from southern Cameroon to southwestern Republic of Congo (35–38). The CIRMF breeding colony was established in 1983/1984 when 15 mandrills (eight females, seven males), originating from the wild, were released into a 6-hectare rainforest enclosure (E1). Increase in group size has been due to natural reproduction of the founder animals, countered by deaths or removal for experimental purposes. In 1994, the colony was separated into two semifree ranging groups (E1 and E2). The mandrills are provisioned twice a day with fruit and monkey chow. Water is available ad libitum. Two hundred and thirty-one infants were born into the colony between June 1983 and June 2002. Blood samples for genetic analysis were collected during annual veterinary captures from 1990 onwards. By 2002, DNA was available for 14 founder animals (one nongenotyped founder female never reproduced) and 205 offspring, from 20 birth seasons (1983–2002; ref. 33).

Reproductive Success. Female reproductive success was defined as the number of offspring surviving >1 year that parous females produced during their reproductive lifespan, using birth seasons from 1983 to 2004. Male reproductive success was defined as the number of offspring sired during birth seasons from 1983 to 2002. Males were considered potentially reproductive from the age of 4 years, by which age the testes have descended (39). We examined both the total number of offspring sired and the number of offspring sired divided by the number of offspring born during the period when the male was potentially reproductive (i.e., correcting for mating opportunity).

Dominance Hierarchies. Rank relations between males and between females were determined by using ad libitum records of avoidance behavior made during daily observation periods. Female dominance ranks are stable in mandrills (40). A female's rank at each birth was expressed as the percentage of all females >3 years of age present that she dominated, to account for demographic changes over time (41). The alpha male was avoided by all other males and never avoided other males. Changes in alpha male identity were clear and occurred from one day to the next.

Genetic Analysis, Relatedness, and Genetic Diversity Estimates. Paternities were assigned by using CERVUS 2.0 (42) and PARENTE (43) software packages. We obtained an accurate paternity assignment for 193 (94%) of the 205 offspring for which blood samples were available (33). Knowledge of the entire colony pedigree allowed calculation of the inbreeding coefficient, f, for each of the 193 offspring. However, calculation of f is based on the assumption that the founder individuals were equally unrelated (32); this is unlikely to be the case, as the founder animals originated from two different areas in Gabon (44) and the colony now represents a mixture of two potentially reproductively isolated populations. Therefore, we calculated relatedness coefficients between founders, by using the Fortran program MOMENT ESTIMATE OF RELATEDNESS (45), to examine whether these coefficients were evenly distributed among individuals coming from the two distinct areas.

Sixty-four males and 73 reproductive females were genotyped for six to eight microsatellite loci (mean ± SEM: 7.68 ± 0.05). Six reproductive, or potentially reproductive, individuals were excluded from the analysis: one male and two females were genotyped at fewer than six loci, and no blood samples were available for a further one male and two females. The exclusion of the two potentially reproductive males from our analysis potentially biased our results because (*i*) they sired no offspring (paternity could be assigned for all offspring born during their potentially reproductive periods) and (*ii*) they did not attain top rank. If the excluded males were highly heterozygous, excluding them would exaggerate any observed trend in the predicted direction. Conversely, if all excluded males showed low heterozygosity, then including them would weaken any observed relationship. However, we have no reason to believe that these males were exceptional in their heterozygosity. Exclusion of four females for whom heterozygosity estimates decreased the sample size, but is unlikely to introduce bias into the data set.

Mean observed heterozygosity per individual (HO) was calculated as the number of heterozygous loci divided by the total number of loci genotyped. HO was 0.82 ± 0.01 (mean ± SEM; n = 137, range 0–1). Internal relatedness (IR) was determined by using the following equation: $(2H - \Sigma fi)/(2N - \Sigma fi)$, where H was the number of homozygous loci, N is the number of loci genotyped, and fi is the frequency of the ith allele contained in the genotype (15). The more an individual is genetically diverse, the more IR will be negative.

Allele frequencies were calculated from the entire data set of 219 individuals rather than the subset of 137 individuals used in the present study. Use of a larger data set reduces the risk of bias due to overrepresentation of rare alleles in a fraction of the population (16).

Statistical Analysis. ***Estimates of genetic diversity and inbreeding coefficients.*** We examined the relationships between f and estimates of genetic diversity in several ways. First, we tested for a correlation between f and the two different estimates of genetic diversity using Spearman correlations (SAS version 9, CORR procedure) and including all individuals typed at six or more loci for which an inbreeding coefficient f was available. We then examined the correlation between relatedness coefficients and the origin of founder individuals using a generalized linear model (SAS version 9, GLM procedure) with a Gaussian error structure, as residuals were normally distributed. We tested *a posteriori* for homoscedasticity between North–North, South–South, and North–South dyads, using Levene's tests, verifying equality of variance. When a correlation between relatedness and founder origin was detected, we used the Lsmean procedure followed by a Tukey test to ordinate the three types of potential pairing. Finally, we tested for a difference in mean heterozygosity between F_1 offspring born to founders from the same area vs. two distinct areas, predicting that animals born to parents from different regions would be more genetically diverse than those born to parents from the same area in Gabon. We used a nonparametric test across a one-way classification (SAS version 9, NPAR1WAY procedure).

Reproductive success. All analyses of reproductive success and dominance rank used generalized linear models (SAS version 9, GENMOD procedure). The error structure and link function

were defined for each model according to the residual distribution of the response variable and following use of exploratory models. Predictor variables were examined for colinearity.

To model the relationship between the number of surviving offspring born to females and genetic diversity (HO and IR), we corrected for female age (in 2004 or at death) and thus for breeding opportunity. We also included a female's mean rank during her lifespan, which is known to influence reproductive success (34). The statistical model used a negative binomial distribution with a log-link and was: Number of surviving offspring = HO or IR + female's age + rank + constant.

The relationship between the number of offspring sired by males and HO or IR was also examined by using a negative binomial distribution. All potentially reproductive males (aged >4 years) were included. We included variable describing whether males were present only as adolescents (aged from 4 to 9 years), because the probability of siring infants is lower in adolescent males than in males that reach adulthood (34), and whether a male attained alpha rank during the study period, as alpha males sired more offspring than subordinates (33, 34). The final statistical model was: Number of offspring sired = HO or IR + adolescent only (Y/N) + alpha (Y/N) + constant.

To examine whether the observed relationship between HO or IR and number of offspring sired was mainly due to alpha or subordinate males (or both), we examined these two sets of males. The number of offspring sired by alpha males was examined using a negative binomial distribution with the following model: Number of offspring sired = HO or IR + constant.

We did not take into account alpha male tenure, because this was itself correlated with genetic diversity (see below).

The number of offspring sired by subordinate males was modeled by using the following equation: Number of offspring sired = HO or IR + adolescent only (Y/N) + constant.

All analyses of male reproductive success were repeated by using the response variable number of offspring sired by each male/number of offspring that he could have sired (see above).

Social rank. To evaluate the relationship between female rank and genetic diversity, we examined females aged >3 years ($n = 72$) for each birth season. Where no change in female rank occurred between two birth seasons, we considered ranks from one birth season only. We used repeated measures, with female identity as the repeated variable, because most females were present for more than one birth season. We used a negative binomial distribution and took matriline identity into account, because females inherit their mother's rank (40), using the following equation: Female rank = HO or IR + matriline + constant.

We used repeated measures logistic regression with a binomial distribution and a logit-link function to examine the relationship between HO or IR and the probability of a male attaining alpha rank, comparing males that became alpha with all other potentially reproductive males present at the time of the takeover. The repeated measure was the identity of any potential future alpha male, because the same males were present during more than one dominance transition. We also included male age, because males attaining alpha status are generally in their prime (39). The final equation was: Became alpha (Y/N) = HO or IR + age + constant.

We repeated this analysis using the sequential number of the dominance transition (categorical variable, range 1–14) as a repeated variable, to remove any effects of specific dominance changes.

Finally, we used a Poisson regression and a log-link function to model the relationship between heterozygosity and the tenure of males who became alpha. We included the mean number of males aged more than five years present during the tenure of a given alpha male to account for the effects of intra-sexual competition, giving: Tenure (years) = HO or IR + number of males + constant.

Contribution of Each Genetic Locus. If a relationship is observed between microsatellite heterozygosity and reproductive success or dominance, this result may be due to either genome-wide heterozygosity at unlinked loci or one or more neutral loci being physically linked to other loci under selection (27, 46). In the first case, the correlation between heterozygosity and fitness should be equivalent across all neutral microsatellite markers. In the second case, the relationship between heterozygosity and fitness will depend mainly on heterozygosity at a single locus (or at a few loci). To investigate whether there were locus-specific effects on fitness, we repeated our analysis fitting each locus individually and dropping one locus at a time from the calculation of genetic diversity (16).

Finally, if heterozygosity reflects inbreeding, then single locus values should be positively correlated across loci. Therefore, we examined the correlation between mean individual heterozygosity in two data sets, each containing four randomly chosen microsatellites of the eight loci analyzed. This analysis was repeated 1,000 times by using SPLUS 2000. A significant "heterozygosity–heterozygosity correlation" indicates a genome-wide effect that is likely to be due to inbreeding, and the higher the correlation, the more precisely heterozygosity reflects inbreeding in the population (9).

Linkage Disequilibrium Between Microsatellite Loci. If large regions of the genome are unaffected by balancing selection, single locus associations may still arise through linkage disequilibrium with other genes affected by selection. In small, or bottlenecked, populations, or where there has been recent mixing between populations, linkage disequilibrium can arise between markers anywhere in the genome, regardless of whether they lie on the same chromosome (47). Because the mandrill colony may represent a mixture of two reproductively isolated populations, we tested for linkage disequilibrium between the eight microsatellite loci used in this study by using FSTAT version 2.9.3.2 (available from http://www2.unil.ch/popgen/softwares/fstat.htm) and sequential Bonferroni corrections for multiple tests (48).

Results

HO and IR were highly correlated ($n = 212$ individuals typed at six or more loci, $r_s = -0.965$, $P < 0.001$). Inbreeding coefficients were also significantly correlated with the two heterozygosity estimates ($n = 212$, $r_{HO\text{-}f} = -0.19$, $P = 0.007$; $r_{IR\text{-}f} = 0.19$, $P = 0.009$). However, the proportion of variance explained by this relationship was low (3.6%). This weak correlation is likely to be explained by the founder animals being unequally unrelated. Indeed, area of origin significantly influenced the relatedness coefficient ($F_{2,88} = 13.81$, $P < 0.0001$), with founders from the same region being significantly more related to one another than those coming from two distinct areas (Fig. 4, which is published as supporting information on the PNAS web site). There was no significant difference in relatedness between North–North and South–South dyads. Finally, the mean heterozygosity of F_1 individuals born to founders from different regions ($n = 44$, mean ± SEM = 0.89 ± 0.02) was significantly greater than that for offspring born to parents from the same area in Gabon ($n = 17$, mean ± SEM = 0.76 ± 0.04; $F_{1,59} = 11.45$; $P = 0.001$). These results suggest that mean heterozygosity estimates (HO and IR) are, as expected, more informative than are inbreeding coefficients obtained from the colony pedigree. In particular, heterozygosity allowed us to distinguish different levels of genetic diversity among individuals where f estimated from the pedigree was zero.

Genetic Diversity and Female Reproductive Success. The number of surviving offspring produced by a female was significantly associated with both estimates of heterozygosity (Table 1): the more heterozygous a female, the more offspring she produced during her lifespan (Fig. 1). Predictably, the number of offspring produced was significantly related to the female's age (with IR: $\chi^2_1 = 96.84$, $P <$

EVOLUTION

Table 1. Associations between HO and IR and reproductive success in females and in males

Dependent variable	*N*	Distribution	HO or IR	Result
Number of surviving offspring (females)	52	Negative binomial	HO	$\chi^2_1 = 7.01$; $P = 0.008$
			IR	$\chi^2_1 = 9.51$; $P = 0.002$
Number of offspring sired by all males	48	Negative binomial	HO	$\chi^2_1 = 4.26$; $P = 0.039$
			IR	$\chi^2_1 = 7.54$; $P = 0.006$
Number of offspring sired by dominant males	9	Negative binomial	HO	$\chi^2_1 = 9.55$; $P = 0.002$
			IR	$\chi^2_1 = 7.97$; $P = 0.005$
Number of offspring sired by subordinate males	39	Negative binomial	HO	$\chi^2_1 = 0.40$; $P = 0.53$
			IR	$\chi^2_1 = 1.11$; $P = 0.29$
Female dominance rank	394 (72 ids)	Negative binomial (repeated measures)	HO	$\chi^2_1 = 0.33$; $P = 0.57$
			IR	$\chi^2_1 = 0.36$; $P = 0.55$
Alpha vs. non-alpha males (repeated individual identity)	194 (59 ids)	Logistic regression (repeated measures)	HO	$\chi^2_1 = 1.10$; $P = 0.29$
			IR	$\chi^2_1 = 0.77$; $P = 0.38$
Tenure of dominant males	12	Poisson regression	HO	$\chi^2_1 = 5.75$; $P = 0.017$
			IR	$\chi^2_1 = 9.18$; $P = 0.003$

0.0001), and reproductive success was also correlated significantly with female rank (with IR: $\chi^2_1 = 4.66$, $P = 0.031$), with higher ranking females giving birth to more offspring. The final statistical model was thus: Number of surviving offspring = −1.15 IR + 0.11 age + 0.003 rank − 0.19.

Genetic Diversity and Male Reproductive Success. The number of offspring sired was significantly associated with both estimates of heterozygosity (Table 1): more heterozygous males sired more offspring (Fig. 2). The number of offspring sired was also significantly associated with whether a male was present as an adult (with IR: $\chi^2_1 = 24.15$, $P < 0.0001$) and whether he attained alpha status (with IR: $\chi^2_1 = 12.41$, $P < 0.001$). The final statistical model was thus: Number of sired offspring = −4.14 IR + 2.83 adolescent phase only − 1.73 dominant male or not − 0.67. These results did not change when the number of offspring sired was replaced with the percentage of offspring sired by all males (for IR: $\chi^2_1 = 5.86$, $P = 0.016$, results not shown for other variables).

Separating alpha from subordinate males, we found that reproductive success was significantly influenced by estimates of heterozygosity in alpha males only (Table 1). More heterozygous alpha males sired more offspring than less heterozygous alpha males (Fig. 2). The final model was: Number of sired offspring = −4.70 IR + 2.06. In subordinate males, only whether a male was present as an adult significantly influenced the number of sired offspring (with IR: $\chi^2_1 = 9.63$, $P = 0.002$). However, the lack of trend among subordinate males could also be ascribed to lack of sufficient variability among individuals. Results were similar when the percentage of offspring sired by dominant males was considered (for IR: $\chi^2_1 = 7.36$, $P = 0.007$).

Genetic Diversity and Dominance Rank. Female rank was not significantly correlated with the estimators of genetic diversity (Table 1), although it was significantly correlated with matriline identity (IR: $\chi^2_6 = 25.43$, $P < 0.001$).

The probability of a male attaining alpha rank was not significantly related to HO or IR (Table 1). Only male age significantly influenced acquisition of alpha status (IR: $\chi^2_1 = 5.05$, $P = 0.025$), with alpha males being older (10.71 ± 0.82 years) than other males present at dominance transitions (8.17 ± 0.28 years). Similar results were found when the sequential number of the dominance transition was included as a repeated variable (data not shown).

Alpha male tenure was significantly influenced by estimators of genetic diversity (Table 1), with more heterozygous males showing longer tenure (Fig. 3). The mean number of males present during the tenure of a given alpha male also correlated significantly with the length of tenure (IR: $\chi^2_1 = 19.66$, $P < 0.0001$). Unsurprisingly, when more males were present, tenure length decreased. The final

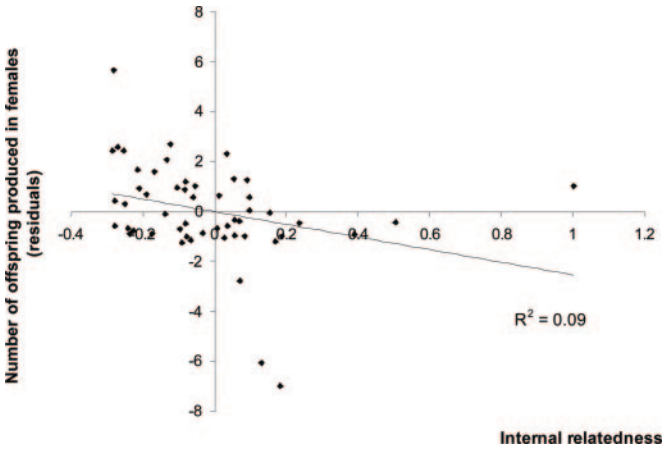

Fig. 1. Genetic diversity and number of offspring produced in females. The figure represents residuals of the number of offspring obtained by using the following equation, plotted against IR: Number of surviving offspring = 0.11 age + 0.004 rank − 0.15.

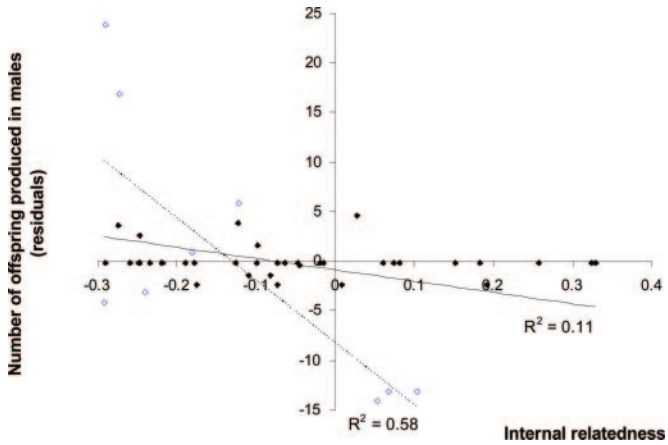

Fig. 2. Genetic diversity and number of offspring sired in males. Filled squares, subordinate males; open squares, alpha males; solid line, regression between the number of offspring sired by all males (including alpha males) and genetic diversity; dashed line, regression between the number of offspring sired by dominant males only and genetic diversity. The figure represents residuals of the number of offspring in all males obtained using the following equation, plotted against IR: Number of sired offspring = 2.81 adolescent phase only − 2.04 alpha male or not + 0.09.

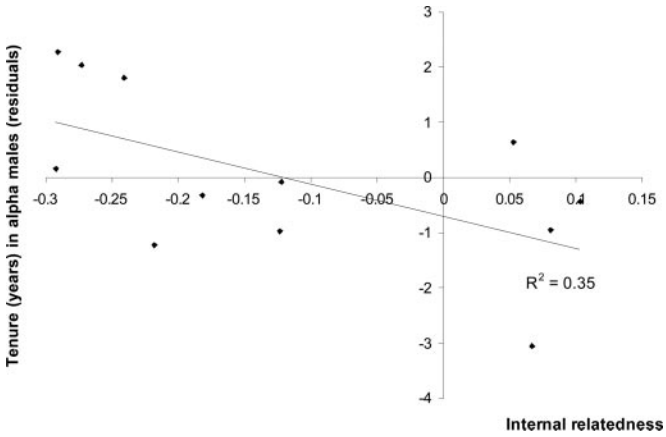

Fig. 3. Genetic diversity and tenure length (residuals) in alpha males. The figure represents residuals of tenure length obtained using the following equation, plotted against IR: Tenure length (years) = −0.14 number of males + 2.60.

statistical model was: Tenure length (years) = −3.42 IR − 0.12 number of males + 1.89.

Contribution of Each Locus. Reanalysis of the relationships between fitness and genetic diversity using each individual locus showed that no particular locus significantly explained variation in either male or female reproductive success, or in alpha male tenure (correcting for multiple tests; Table 2, which is published as supporting information on the PNAS web site). This analysis was not repeated for alpha males only due to the small sample size ($n = 9$).

Dropping one locus at a time from the calculation of IR did not alter the influence of IR on female reproductive success or on tenure length of alpha males (correcting for multiple tests; Table 3, which is published as supporting information on the PNAS web site). The removal of three loci resulted in only a marginally significant relationship between IR and male reproductive success, whereas the removal of five other loci resulted in a significant relationship (Table 3).

Finally, comparison of mean heterozygosity between two data sets ($n = 141$ individuals typed at eight loci), each containing four of the eight loci analyzed, showed a significant correlation between the two data sets in 87.6% of simulated cases. The average correlation coefficient over 1,000 simulations was $r = 0.23$. Although this correlation coefficient is not high, this result suggests that our eight markers are representative of genome-wide heterozygosity.

Linkage Disequilibrium Between Microsatellite Loci. Of 28 pairwise associations between loci, only two showed nonrandom association at the 5% level, and only one of these was significant at the 1% level after sequential Bonferroni correction (Table 4, which is published as supporting information on the PNAS web site).

Discussion

We examined a large data set concerning the demography and genetic diversity of a semifree ranging population of mandrills to investigate whether two estimates of heterozygosity were related to reproductive success and/or social rank in males and females. We also examined whether any relationships between these variables were associated with genome-wide heterozygosity among unlinked genes, or were due to neutral loci being physically linked to loci under selection pressures (see ref. 27). As in studies of other mammals (11, 14–25), we found that HO and IR were highly correlated, and results were similar for the two heterozygosity estimates, with IR exhibiting the higher correlation with fitness. In both males and females, individuals with more heterozygous microsatellite loci had the highest reproductive success, as measured by the number of offspring produced. Finally, genetic diversity was not related to dominance rank in either sex, although more heterozygous alpha males enjoyed longer tenure. Our findings support the hypothesis that the correlation between heterozygosity and fitness is due to inbreeding depression at a genome-wide level, rather than being driven by only a few loci.

The correlation between heterozygosity and reproductive success may be due to increased metabolic efficiency (49) and/or to disease resistance (21, 50) in heterozygous animals, allowing them to better afford the cost of reproduction (raising offspring in females, intrasexual competition in males) than homozygous animals. For example, disease resistance may be the consequence of heterozygosity at the immunologically important MHC (51). A recent study of semifree-ranging rhesus macaques found that males heterozygous at one moderately variable MHC locus sired more offspring than more homozygous males (52). Interestingly, although our results imply a genome-wide inbreeding effect, in the rhesus study, males that were homozygote and heterozygote at the MHC locus did not differ in microsatellite heterozygosity, ruling out a genome-wide inbreeding effect.

The CIRMF population of mandrills is relatively small, with no gene flow, and is characterized by a strongly polygynous breeding system in which inbreeding effects are likely to be particularly manifest (27). Although nothing is known about the genetic diversity of wild mandrill populations, it is possible that less extreme breeding systems and less isolated populations experience little, or no, inbreeding depression, as all males may disperse from their natal group. However, although the colony studied here may represent an extreme situation, it is also possible that at least some male mandrills sire offspring in their natal group because males are sexually mature at 4 years and may sire offspring before dispersal, which occurs at ≈6 years (34, 37, 39). The specific conditions of the CIRMF colony represent an experimental setting, allowing the study of evolutionary phenomena that may have influenced mandrills in the wild. For example, past inbreeding depression may have led to the evolution of the incest avoidance mechanisms present in wild populations today (3, 4). Finally, such restricted conditions may provide a useful model for populations undergoing habitat fragmentation in the wild (53).

Our finding that female rank is unrelated to genetic diversity is unsurprising because female rank is inherited matrilinearly in mandrills (40) and is therefore less likely to be influenced by genetic "quality" than is rank in males, which is determined by contest competition. Male–male competition is intense in mandrills, suggesting that heterozygous (and thus metabolically more efficient, or more disease-resistant) males should be at an advantage. Studies of other species have shown that less heterozygous males are disadvantaged in male–male competition. For example, in black grouse (*Tetrao tetrix*), males that never obtained a lek territory had significantly lower mean heterozygosity than males that were observed on a territory during at least one mating season (54). Heterozygosity was also linked to territory size in the subdesert mesite (*Monias benschi*; ref. 24), and highly heterozygous male Antarctic fur seals (*Arctocephalus gazella*) are more successful in male–male competition than less heterozygous males (16).

Alpha male mandrills experience high social pressures, both from competitive subordinates and from females (55), and the positive relationship between tenure as dominant male and heterozygosity suggests that heterozygous males can afford the costs of the alpha position for a longer period. The lack of a significant relationship between heterozygosity and the probability of gaining alpha rank in males may be a consequence of relatively few homozygous males having attained the age at which they might reach alpha rank. Reduced reproductive success in less heterozygous dominant males suggests that sneak copulations with subordinates may occur more often if an alpha male is of poor genetic quality. However, het-

erozygous subordinate males did not experience a reproductive advantage, suggesting that more heterozygous subordinates are not more successful in sneaky reproduction.

The greater reproductive success observed in heterozygous alpha males may be a by-product of a longer tenure. Alternatively, females may preferentially choose more heterozygous alpha males as partners, to gain resources or genetic benefits for their offspring (56). Similarly, males may show choice for genetically fitter females. Both male and female mandrills show elaborate secondary sexual ornaments: bright coloration in males and sexual swellings in females (57). Our finding that reproductive success is associated with heterozygosity in mandrills raises the question of whether these traits honestly advertise genetic quality ("good genes," refs. 58–60) in the form of heterozygosity. Female mandrills prefer to mate with brightly colored males, reinforcing the effects of male–male competition on male reproductive success (61). It remains to be seen whether brightly colored males are genetically more diverse. In females, swelling size may advertise genetic diversity. A controversial study of baboons found that males preferred to mate with females possessing larger sexual swellings, which signaled increased female reproductive success (62, 63). However, we were not able to replicate these findings for this colony of mandrills, where male mate choice is unrelated to the size of female sexual swellings and swelling size is unrelated to female reproductive quality (64).

To date, studies examining inbreeding depression in primate species have examined only maternal relationships, obtained from behavioral observations, or concern very small populations (6, 65–67). However, our study used genetic diversity estimates to demonstrate that inbreeding depression can negatively influence reproductive success in a primate population.

We are grateful to the Centre International de Recherches Médicales de Franceville (CIRMF) for permission to study the mandrill colony, and for logistical support. We thank Simon Ossari for valuable technical help; Philippe Jarne, Doyle McKey, and Marc Choisy for helpful advice and comments on the manuscript; and Philippe Blot (Director General, CIRMF) for support. We are grateful to two anonymous referees for helpful comments on a previous version of the manuscript. The CIRMF is financed by the Gabonese government, Total Gabon, and the Coopération Française. The mandrill studies presented here were funded by National Institute of Allergy and Infectious Diseases Grant 5 R01 AI44596 (to Dr. Preston Marx, Tulane University, Houston), the Ministère Français des Affaires Etrangères, and a Leverhulme Trust, U.K. Project Grant F/01576/B.

1. Darwin, C. (1876) *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom* (John Murray, London).
2. Charlesworth, D. & Charlesworth, B. (1987) *Annu. Rev. Ecol. Syst.* **18,** 237–268.
3. Brooker, M. G., Rowley, I., Adams, M. & Baverstock, P. R. (1990) *Behav. Ecol. Sociobiol.* **26,** 191–199.
4. Clutton-Brock, T. H. (1989) *Nature* **337,** 70–72.
5. Frankham, R. (1998) *Conserv. Biol.* **12,** 665–675.
6. Lacy, R. C., Petric, A. & Warneke, M. (1993) in *The Natural History of Inbreeding and Outbreeding*, ed. Thornhill, N. W. (Univ. of Chicago Press, Chicago), pp. 352–374.
7. Falconer, D. S. & Mackay, T. F. C. (1996) *Introduction to Quantitative Genetics* (Longman, Essex, U.K.).
8. Lynch, M. (1998) *Genetics and Analysis of Quantitative Traits* (Sinauer, Sunderland, MA).
9. Pemberton, J. (2004) *Trends Ecol. Evol.* **19,** 613–615.
10. Hartl, D. L. & Clark, A. G. (1997) *Principles of Population Genetics* (Sinauer, Sunderland, MA).
11. Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M. A., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. (1998) *Proc. R. Soc. London Ser. B* **265,** 489–495.
12. Slate, J. & Pemberton, J. M. (2002) *J. Evol. Biol.* **15,** 20–31.
13. Tsitrone, A., Rousset, F. & David, P. (2001) *Genetics* **159,** 1845–1859.
14. Coltman, D. W., Pilkington, J. G., Smith, J. A. & Pemberton, J. M. (1999) *Evolution (Lawrence, Kans.)* **53,** 1259–1267.
15. Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. (2001) *Proc. R. Soc. London Ser. B* **268,** 2021–2027.
16. Hoffman, J. I., Boyd, I. L. & Amos, W. (2004) *Evolution (Lawrence, Kans.)* **58,** 2087–2099.
17. Coulson, T. N., Albon, S. D., Slate, J. & Pemberton, J. M. (1999) *Evolution (Lawrence, Kans.)* **53,** 1951–1960.
18. Crnokrak, P. & Roff, D. A. (1999) *Heredity* **83,** 260–270.
19. Slate, J., Kruuk, L. E. B., Marshall, T. C., Pemberton, J. M. & Clutton-Brock, T. H. (2000) *Proc. R. Soc. London Ser. B* **267,** 1657–1662.
20. Keller, L. & Waller, D. M. (2002) *Trends Ecol. Evol.* **17,** 230–241.
21. Acevedo-Whitehouse, K., Gulland, F., Grieg, D. & Amos, W. (2003) *Nature* **422,** 6227.
22. Gallardo, J. A., Garcia, X., Lhorente, J. P. & Neira, R. (2004) *Aquaculture* **234,** 111–122.
23. Mandal, A., Pant, K. P., Rout, P. K. & Roy, R. (2004) *Asian-Australian J. Anim. Sci.* **17,** 594–597.
24. Seddon, N., Amos, W., Mulder, R. A. & Tobias, J. A. (2004) *Proc. R. Soc. London Ser. B* **271,** 1823–1829.
25. Pujolar, J. M., Maes, G. E., Vancoillie, C. & Volckaert, F. A. M. (2005) *Evolution (Lawrence, Kans.)* **59,** 189–199.
26. Britten, H. B. (1996) *Evolution (Lawrence, Kans.)* **50,** 2158–2164.
27. Balloux, F., Amos, W. & Coulson, T. (2004) *Mol. Ecol.* **13,** 3021–3031.
28. Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E. & McEwan, J. C. (2004) *Heredity* **93,** 255–265.
29. Coltman, D. W. & Slate, J. (2003) *Evolution (Lawrence, Kans.)* **57,** 971–983.
30. Hansson, B., Westergahl, H., Hasselquist, D., Åkesson, M. & Bensch, S. (2004) *Evolution (Lawrence, Kans.)* **58,** 870–879.
31. Markert, J. A., Grant, P. R., Grant, B. R., Keller, L. F., Coombs, J. L. & Petren, K. (2004) *Heredity* **92,** 306–315.
32. Wickings, E. J. (1995) *Electrophoresis* **16,** 1678–1683.
33. Charpentier, M., Peignot, P., Hossaert-McKey, M., Gimenez, O., Setchell, J. M. & Wickings, E. J. (2005) *Behav. Ecol.* **16,** 614–623.
34. Setchell, J. M., Charpentier, M. & Wickings, E. J. (2005) *Behav. Ecol. Sociobiol.* **58,** 467–485.
35. Harrison, M. J. S. (1988) *Oryx* **22,** 218–228.
36. Grubb, P. (1973) *Folia Primatol.* **20,** 161–177.
37. Abernethy, K. A., White, L. J. T. & Wickings, E. J. (2002) *J. Zool.* **258,** 131–137.
38. Rogers, M. E., Abernethy, K. A., Fontaine, B., Wickings, E. J., White, L. J. T. & Tutin, C. E. G. (1996) *Am. J. Primatol.* **40,** 297–313.
39. Setchell, J. M. & Dixson, A. F. (2002) *Am. J. Primatol.* **56,** 9–25.
40. Setchell, J. M. (1999) Ph.D. dissertation (Univ. of Cambridge, Cambridge, U.K.).
41. Cheney, D. L., Seyfarth, R. M., Andelman, S. J. & Lee, P. C. (1988) in *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems*, ed. Clutton-Brock, T. H. (Univ. of Chicago Press, Chicago), pp. 384–402.
42. Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. (1998) *Mol. Ecol.* **7,** 639–655.
43. Cercueil, A., Bellemain, E. & Manel, S. (2002) *J. Hered.* **93,** 458–459.
44. Telfer, P. T., Souquière, S., Clifford, S. L., Abernethy, K. A., Bruford, M. W., Disotell, T. R., Sterner, K. N., Roques, P., Marx, P. A. & Wickings, E. J. (2003) *Mol. Ecol.* **12,** 2019–2024.
45. Wang, J. L. (2002) *Genetics* **160,** 1203–1215.
46. Hansson, B. & Westerberg, L. (2002) *Mol. Ecol.* **11,** 2467–2474.
47. Ohta, T. & Kimura, M. (1970) *Genet. Res.* **16,** 165–177.
48. Rice, W. R. (1989) *Evolution (Lawrence, Kans.)* **43,** 223–225.
49. Mitton, J. B. (1993) *Genetica* **89,** 47–65.
50. Aparicio, J. M., Cordero, P. J. & Veiga, J. P. (2001) *Anim. Behav.* **62,** 1001–1006.
51. Edwards, S. V. & Hedrick, P. W. (1998) *Trends Ecol. Evol.* **13,** 305–311.
52. Sauermann, U., Nürnberg, P., Bercovitch, F. B., Berard, J. D., Trefilov, A., Widdig, A., Kessler, M., Schmidtke, J. & Krawczak, M. (2001) *Hum. Genet.* **108,** 249–254.
53. Frankham, R. (1995) *Annu. Rev. Genet.* **29,** 305–327.
54. Höglund, J., Piertney, S. B., Alatalo, R. V., Lindell, J., Lundberg, A. & Rintamaki, P. T. (2002) *Proc. R. Soc. London Ser. B* **269,** 711–715.
55. Setchell, J. M., Knapp, L. A. & Wickings, E. J. (2005) *Am. J. Primatol.*, in press.
56. Mays, H. L. J. & Hill, G. E. (2004) *Trends Ecol. Evol.* **19,** 554–559.
57. Hill, W. C. O. (1970) *Primates, Comparative Anatomy and Taxonomy, Vol 8, Cynopithecinae, Papio, Mandrillus, Theropithecus* (Edinburgh Univ. Press, Edinburgh, U.K.).
58. Hamilton, W. D. & Zuk, M. (1982) *Science* **218,** 384–387.
59. Zahavi, A. (1975) *J. Theor. Biol.* **53,** 205–214.
60. Andersson, M. (1994) *Sexual Selection* (Princeton Univ. Press, Princeton).
61. Setchell, J. M. (2005) *Int. J. Primatol.* **26,** 713–732.
62. Domb, L. G. & Pagel, M. (2001) *Nature* **410,** 204–206.
63. Zinner, D., Alberts, S. C., Nunn, C. L. & Altmann, J. (2002) *Nature* **420,** 142–143.
64. Setchell, J. M. & Wickings, E. J. (2003) *Behav. Ecol.* **15,** 438–445.
65. Moore, J. (1993) in *The Natural History of Inbreeding and Outbreeding*, ed. Thornhill, N. W. (Univ. of Chicago Press, Chicago), pp. 392–426.
66. Smith, D. G. (1995) *Int. J. Primatol.* **16,** 855–870.
67. Charpentier, M., Hossaert-McKey, M., Wickings, E. J. & Peignot, P. (2005) *Int. J. Primatol.* **26,** 697–710.