

Secondary sexual characters and female quality in primates

Joanna M. Setchell · Marie J. E. Charpentier ·
Issa-Ben Bedjabaga · Patricia Reed · E. Jean Wickings ·
Leslie A. Knapp

Received: 31 March 2006 / Revised: 12 July 2006 / Accepted: 13 July 2006
© Springer-Verlag 2006

Abstract Honest advertisement models of sexual selection propose that exaggerated secondary sexual ornaments are condition-dependent, and that only individuals with superior disease resistance will be able to express costly ornamentation. Studies of secondary sexual ornamentation and their maintenance by sexual selection tend to focus on males. However, females may also possess showy ornaments. We investigated whether female ornaments, in the form of sexual swellings, reliably signal female fitness in a semifree-ranging colony of mandrills (*Mandrillus*

sphinx) at the Centre International de Recherches Médicales, Franceville (CIRMF), Gabon. We measured swelling height and width using photographs of periovulatory females over three mating seasons and compared swelling size with parasitism (using fecal analysis over one annual cycle), immune status (ratio of lymphocytes to neutrophils in blood smears made during captures), and genetic diversity (microsatellite heterozygosity). Swelling size varied by up to 10% between cycles in individual females, giving some support to the hypothesis that size differences may indicate the quality of individual swelling cycles. However, there was no significant difference in swelling size between conceptive and nonconceptive cycles. Measures of swelling size varied more between females than within females across swelling cycles, implying that swelling size was a relatively consistent characteristic of individual females. Swelling size was not significantly related to either general measures of parasitism and immune status, or to the closest available measures to each swelling cycle. Nor was swelling size significantly related to genetic diversity. The healthy, provisioned nature of the colony and problems associated with observational, correlational studies restrict interpretation of our data. However, in combination with previous findings that females of higher reproductive success do not show larger swellings, and that males do not allocate mating effort as a function of swelling size, these results imply that sexual swelling size does not indicate female quality in these semifree-ranging mandrills.

Communicated by C. Nunn

J. M. Setchell (✉) · L. A. Knapp
Department of Biological Anthropology,
University of Cambridge,
Downing Street,
Cambridge CB2 3DZ, UK
e-mail: mandrills@yahoo.co.uk

J. M. Setchell · M. J. E. Charpentier · E. J. Wickings
Centre International de Recherches Médicales,
BP 769, Franceville, Gabon

M. J. E. Charpentier
Centre d'Ecologie Fonctionnelle et Evolutive UMR 5175, CNRS,
1919 Route de Mende,
34293 Montpellier Cedex 5, France

M. J. E. Charpentier
Duke University,
Science Drive, Biological Building 020, P.O. 90338,
Durham, NC 27708, USA

I.-B. Bedjabaga · P. Reed
Field Veterinary Program, Wildlife Conservation Society,
New York, NY, USA

Keywords Hamilton and Zuk hypothesis · Sexual selection · Parasites · Immune status · Heterozygosity · Secondary sexual characters

Introduction

Exaggerated sexual characters are generally thought to be costly to develop and maintain, and therefore to require a selective advantage to explain their existence. Honest advertisement models of sexual selection propose that exaggerated secondary sexual ornaments are condition-dependent, and that only individuals of superior quality will be able to express costly ornamentation (Zahavi 1975). The Hamilton and Zuk hypothesis suggests that secondary sexual ornaments may reliably reflect ability to resist parasites by revealing current health status (Hamilton and Zuk 1982). In particular, ornaments may signal the current ability of the bearer to raise an immune response toward parasites (Møller and Saino 1994). Members of the opposite sex will be selected to choose the most ornamented mate because such mates provide fitness benefits. These benefits may be direct, in terms of parasite transmission avoidance and/or increased investment in offspring, or indirect, such as “good genes” for vigor and health (Zahavi 1975; Hamilton and Zuk 1982; Andersson 1994; Able 1996). Candidates for such good genes include possession of specific genes for resistance to various pathogens, such as major histocompatibility complex (MHC) genes (von Schantz et al. 1996). Alternatively, exaggerated ornaments may signal a high level of average heterozygosity (Brown 1997). High heterozygosity is associated with increased survival and fitness (Keller and Waller 2002), probably because it reduces the likelihood that recessive deleterious alleles will be expressed (Charlesworth and Charlesworth 1987) and increases the number of potentially useful gene products, including MHC genes (Mitton et al. 1993; von Schantz et al. 1996; Edwards and Hedrick 1998; Penn and Potts 1999; Jennions and Petrie 2000).

Evidence that exaggerated ornament expression is related to parasite infection and/or immune status now exists for many species (reviews in Zuk 1992; Andersson 1994; Johnstone 1995; Møller et al. 1999). Ornament expression correlates positively with genetic diversity, or negatively with inbreeding, in male birds (Aparicio et al. 2001; Foerster et al. 2003; Marshall et al. 2003), fish (Müller and Ward 1995; Sheridan and Pomiankowski 1997; van Oosterhout et al. 2003), and mammals (Scribner et al. 1989). Genetic diversity at specific loci (e.g., MHC) is also significantly associated with variation in ornamentation in birds (von Schantz et al. 1996, 1997) and mammals (Ditchkoff et al. 2001). However, although females may also possess showy ornaments, studies of relationships between ornament expression and fitness have tended to focus on males (Amundsen 2000).

Females of many Old World primate species develop conspicuous swellings of the perineal skin that reach maximum size around the time of ovulation. These “sexual

swellings” have evolved independently in at least three separate primate lineages and are associated with multi-male–multifemale mating systems (Dixson 1983). They are hormone-dependent (Dixson 1998), attract males for mating independently of olfactory or behavioral cues (Bielert and van der Walt 1982; Bielert and Anderson 1985; Bielert and Girolami 1986; Bielert et al. 1989; summarized in Snowdon 2004), and are likely to be costly to the females (Nunn et al. 2001). A number of hypotheses, not necessarily mutually exclusive, have been proposed to account for the evolution of sexual swellings (reviewed by Nunn 1999; Stallmann and Froehlich 2000). However, few studies have tested the predictions of these hypotheses and the exact function of sexual swelling remains unclear and controversial (Domb and Pagel 2001, 2002; Zinner et al. 2002).

Females are expected to be the choosy sex in primates because parental investment is strongly biased toward females through the costs of gestation and lactation. However, males should also be choosy if females vary greatly in quality, or if mating with one female reduces a male’s chances of mating with other females (Andersson 1994). For example, primate males that attempt to monopolize access to one receptive female may miss the opportunity to mate with another female (Altmann 1962). They also risk injury in contest competition (Drews 1996) and face constraints on foraging activity, which are likely to result in decreased energy intake (Bercovitch 1983; Alberts et al. 1996). Moreover, sperm production is costly and sperm delivery and ejaculate quality are compromised by successive ejaculations (Dewsbury 1982; Marson et al. 1989; Preston et al. 2001; Wedell et al. 2002). To maximize their reproductive success males should show mate choice. They should apportion costly mating effort in relation to the quality of an individual female and cycle, and preferentially compete for the most fertile females and those that will produce the highest quality offspring. The “reliable indicator hypothesis” proposes that sexual swellings in primates can be regarded as a costly handicap that honestly signals female quality to males (Pagel 1994). Males are hypothesized to choose females with larger swellings because such females are more fertile and will produce higher quality offspring.

A recent field study on olive baboons (*Papio cynocephalus anubis*) in Gombe National Park, Tanzania (Domb and Pagel 2001) found support for the reliable indicator hypothesis. Female baboons with larger (in one of the three dimensions measured) swellings attained sexual maturity earlier, produced more offspring, and produced more surviving offspring. Male baboons showed greater interest in, and competed more intensely for, females with larger swellings. However, Zinner et al. (2002) have highlighted several flaws in this study. Specifically, Domb and Pagel (2001) ignored intraindivid-

ual variability of swelling characteristics, the possibility that the covariate body size could explain the significant results, and variation in demography and food availability between the five baboon groups studied. Synchronously mating females were also excluded from the analysis, although investigation of overlapping estrus cycles might be expected to show the strongest patterns of male mate choice for large swellings (Zinner et al. 2002). Furthermore, lifetime reproductive parameters are not necessarily of most interest to choosy mates, and swelling size may indicate other aspects of female fitness, such as resistance to parasites, general health status, or genetic quality. Finally, variation in swelling size might serve as an indicator of the quality of an individual cycle, and thus a female's proximate reproductive quality (Zinner et al. 2002; Emery and Whitten 2003).

In this study, we investigate relationships between swelling size and female fitness in terms of individual cycle quality, parasitism, general immune status, and genetic diversity in a semifree-ranging colony of mandrills, housed at CIRMF. Mandrills are found only in the dense rainforest of central Africa (Gabon, Republic of Congo, Equatorial Guinea, and Cameroon; Grubb 1973). They are thought to travel in large, semidisaggregated bands in which males may not have direct knowledge of a female's quality (Abernethy et al. 2002). A quality indicator may therefore be more important for this species than for other primates, such as macaques or baboons, in which males and females have more stable, longer term relationships. Forest conditions entail that mandrills are extremely difficult to study and they have so far proved impossible to habituate in the wild (Harrison 1988; Abernethy et al. 2002). Most of our knowledge of mandrill reproduction and behavior therefore comes from the CIRMF colony. A previous study of this colony showed that swelling size is not related to female reproductive quality (Setchell and Wickings 2004a), but this does not preclude the possibility that swelling size reflects other fitness parameters in females.

We used photographic methods to measure sexual swelling size. We examined cycle-to-cycle variability in swelling size and compared conceptive and non-conceptive cycles for the same females. To compare swelling size with female fitness, we estimated parasite infection via fecal parasite analysis and general immune status using the ratio of neutrophils to lymphocytes. An increase in this ratio indicates a reduction in immune function and is related to stress in primates (e.g., Kim et al. 2005). Genetic diversity was examined using internal relatedness (IR; Amos et al. 2001). IR provides a good measure of genome-wide inbreeding in this population, and is more informative than inbreeding coefficients calculated from the colony pedigree, as the

founder animals were not equally unrelated (Charpentier et al. 2005). If female swelling size acts as a reliable signal of cycle quality (Zinner et al. 2002; Emery and Whitten 2003), we then predicted that swellings of nonconceptive cycles should be smaller than those of conceptive cycles. If swelling size reliably indicates female fitness in terms of parasite resistance (Hamilton and Zuk 1982), then females with low parasitism should show larger swellings than females with high parasitism. Similarly, if swelling size indicates immune system quality (Moller and Saino 1994), then females with better than average general immune status should show larger swellings than those with lower than average general immune status. Finally, if swelling size indicates heterozygosity (Brown 1997), then females with greater heterozygosity should show larger swellings than females with lower heterozygosity.

Materials and methods

Study animals

The CIRMF mandrill colony was established in 1983/1984 when 15 animals (seven males and eight females) were released into a 6.5-ha forest enclosure. There have been no subsequent additions to the colony other than by breeding, although animals are occasionally removed, and in 1994 a second semifree-ranging group was established in a smaller enclosure (3.5 ha) by transferring animals from the first enclosure. E1 housed 106, 69, and 83 animals during the mating seasons in 2002, 2003, and 2004, respectively. E2 housed 52, 62, and 72 animals. The animals forage freely and receive daily supplements of monkey chow, fruit, and vegetables. Water is always available from a stream, which runs through both enclosures.

Daily records were kept of the swelling status of all females in the colony. This study involved 32 of the 33 parous females that showed estrus cycles with swellings during the 2002–2004 mating seasons (no suitable photographs were available for the remaining female). Subjects were members of seven different founder matriline (1–10 females per matriline), had an age range of 4.4–24.0 years when they first contributed data, and a parity of 1–20 previous offspring. Nulliparous females were not included, as their swellings are significantly smaller than those of parous females (Setchell and Wickings 2004a). Swelling size does not correlate with age (Setchell and Wickings 2004a). The conceptive or non-conceptive nature of each swelling cycle was determined 3 months after cycles, when females were noticeably pregnant and showed pregnancy swellings.

Quantifying swelling size

The size of the maximal swelling for each cycle for each female was measured using still photographs (2002) or digital images (2003–2004) of females from behind and from the side, taken at close range during daily provisioning. Only images obtained during the periovulatory period (last 5 days of maximal swelling; Wildt et al. 1977; Shaikh et al. 1982) were used. Suitable photographs were scanned or downloaded into a computer and analyzed using Scion Image for Windows (available at <http://www.scion.com>). Images were scaled using one or (preferably) more of the following: the height and width of the ischial callosities, the length and width of the tail, and standing height (all measured at capture). For each swelling we recorded the height (top to bottom, rear or side view), maximum width (rear view), and maximum depth (side view). Measures made using this method do not vary more than 5% between photographs of the same swelling cycle, and are within 5% of measures taken on anesthetized females (Setchell and Wickings 2004a).

Measures of swelling height and width were significantly correlated ($r=0.641$, $p<0.001$, $n=88$ cycles). Swelling depth was also positively correlated with both height ($r=0.353$, $p=0.001$, $n=92$ cycles) and width ($r=0.387$, $p<0.001$, $n=82$ cycles), but was the least satisfactory measure as it was the most variable between photographs of the same female on the same day. We therefore analyzed swelling height and width only, using both absolute measures of swelling size, and swelling size corrected for female standing height (Zinner et al. 2002). Standing height varied between 36 and 48.5 cm in this sample of females (mean \pm SEM 41.6 \pm 0.7 cm), and was positively and significantly linearly related to both swelling height ($r=0.517$, $p=0.002$, $n=32$) and width ($r=0.432$, $p=0.017$, $n=30$).

Quantifying parasitism

When possible, one to three fecal samples were collected per month for all parous females between April 2004 and March 2005. Samples were collected immediately after defecation during morning (1000–1130 hours) or afternoon (1530–1730 hours) observation periods. Feces were homogenized and a portion (mean \pm SEM 7.6 \pm 0.1 g) was stored in 20 ml of 10% formalin solution until analysis by an independent parasitologist who had no knowledge of the individuals concerned. Samples were examined using direct smears and centrifugation/flotation using a Sheather's solution at a specific gravity of 1.18. Parasitic eggs, larvae, trophs, and cysts were recorded by species according to characteristic morphology, and were quantified as 0 (none), 1 (1–5), 2 (6–10), and 3 (more than 10).

We quantified general levels of parasitism for the ten females that showed sexual swellings during the sampling period, and for whom samples were available for at least 6 of the 12 months studied (to avoid any systematic bias) as:

- (1) Mean monthly abundance of cysts or eggs of each taxon in the feces (scale of 0–3 above)
- (2) Mean monthly presence of each taxon (number of samples in which a particular taxon occurred divided by the number of samples, expressed as a percentage)
- (3) Mean monthly parasite diversity (number of taxa found in the feces)

We also measured parasitism at the time of each swelling cycle using samples from the closest sampling month for females for whom three samples were available (the number of taxa detected increases with the first few samples collected; Setchell et al. unpublished data) as follows:

- (4) Presence/absence of each taxon in the feces
- (5) Abundance of each taxon in the feces
- (6) Parasite diversity (number of taxa found in the feces)

Quantifying general immune status

The mandrills were captured approximately annually for veterinary examination. Anesthesia was accomplished using a "Telinject" blowpipe to deliver a syringe containing "Imalgene1000" (10 mg/kg, Rhone-Mérieux, Lyon, France). Blood samples were taken via venipuncture approximately 20 min after anesthesia. Seven milliliters of blood was collected from the femoral vein into vacutainer tubes containing EDTA. One milliliter of this sample was then transferred into a separate tube for hematological analyses, which were conducted by the Laboratoire des Analyses Médicales (LAM) at CIRMF (details in Setchell et al. 2006). Differential leukocyte counts of EDTA were obtained using dried blood smears fixed with methanol and stained with Wright's Giemsa stain.

We measured general immune status as the ratio of neutrophils to lymphocytes. This was corrected for age and year of capture using standardized residuals from a univariate analysis of variance for all data available for females in the colony, as both variables are known to influence hematological parameters (Setchell et al. 2006). We used the mean of all values available for each individual (1–19 data points per individual, mean 4.0 \pm 0.6) as a measure of general health, and values from the closest capture to each swelling cycle as an estimate of health at the time of that cycle.

Quantifying genetic diversity

DNA was available for all study subjects from blood samples collected during annual veterinary captures. Individuals were genotyped for six to eight microsatellite loci (mean±SEM 7.73±0.10). IR was determined using the following equation:

$$\frac{(2H - \sum f_i)}{(2N - \sum f_i)}$$

where H was the number of homozygous loci, N the number of loci genotyped, and f_i the frequency of the i th allele contained in the genotype (Amos et al. 2001). The more genetically diverse an individual was, the more negative the IR. Allele frequencies were calculated from the entire data set of 219 individuals. Use of a larger dataset reduces the risk of bias due to overrepresentation of rare alleles in a fraction of the population (Hoffman et al. 2004).

Despite the small founder population and lack of immigration in the CIRMF mandrill colony, inbreeding was unlikely to affect the results of our study. Five of the study females were founders (inbreeding coefficient (IC) assumed to be zero), 15 were first generation (IC=0), and six others belonged to subsequent generations but their IC (calculated from the known pedigree) was 0. Of the remaining six study subjects, five had an IC of 0.125 and one had an IC of 0.25. These females did not show swellings that were significantly different in size to those of noninbred females (data not shown).

Statistical analyses

We investigated whether swelling characteristics were consistent characteristics of individual females using analysis of variance, with swelling size as the dependent variable, and female ID as a factor. If swelling characteristics are consistent across cycles for individual females, then within-female variance should be smaller than between-female variance, and we should find a significant influence of female ID on swelling size. Where females exhibited multiple cycles to conception, we compared swelling size in the conceptive cycle with that during the previous cycle. Measures of prior cycles were available for only two females, precluding statistical comparison of multiple nonconceptive cycles.

We used bivariate Pearson's correlations to compare swelling measures with measures of parasitism, immune status, and genetic diversity. A priori predictions (hypothesized positive relationships between swelling size and other variables) were tested using one-tailed significance levels, denoted p_1 ; all other tests are reported with two-tailed significance levels, p_2 . Tests where $p < 0.05$ are reported as significant. We present unadjusted significance

levels, and the sequential Bonferroni threshold in tables of results (Rice 1989). Post hoc power analyses were calculated using GPOWER (Erdfelder et al. 1996). Sample sizes vary as not all measures were available for all females.

Results

Consistency of swelling size

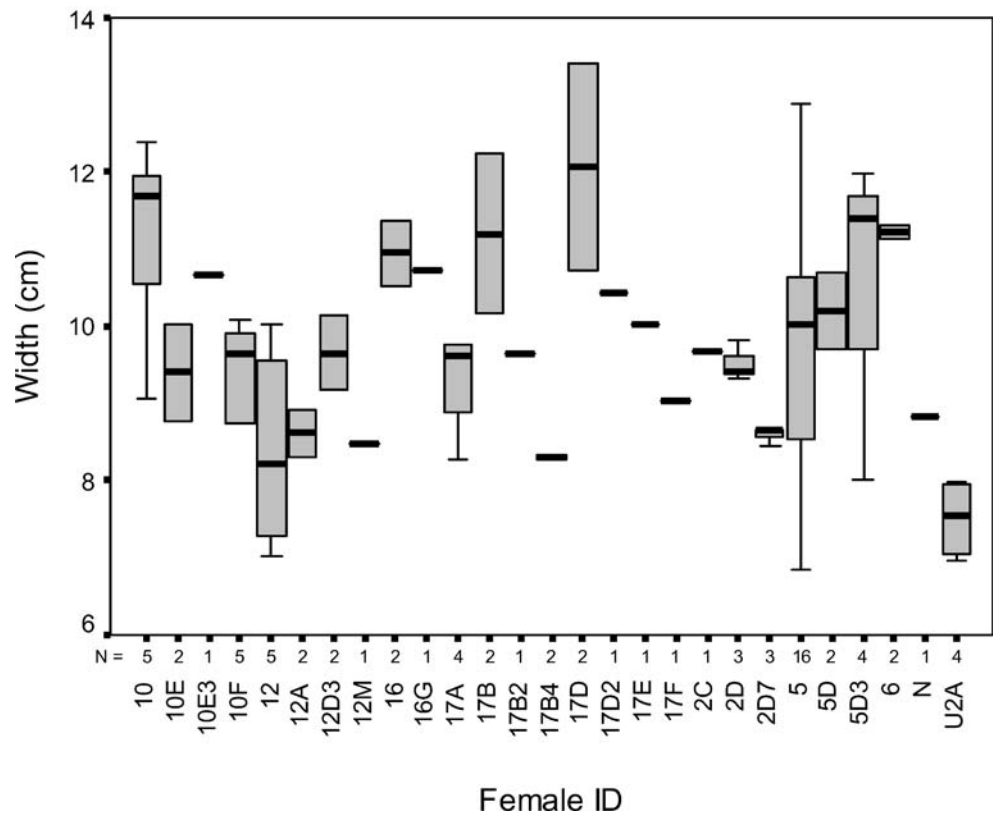
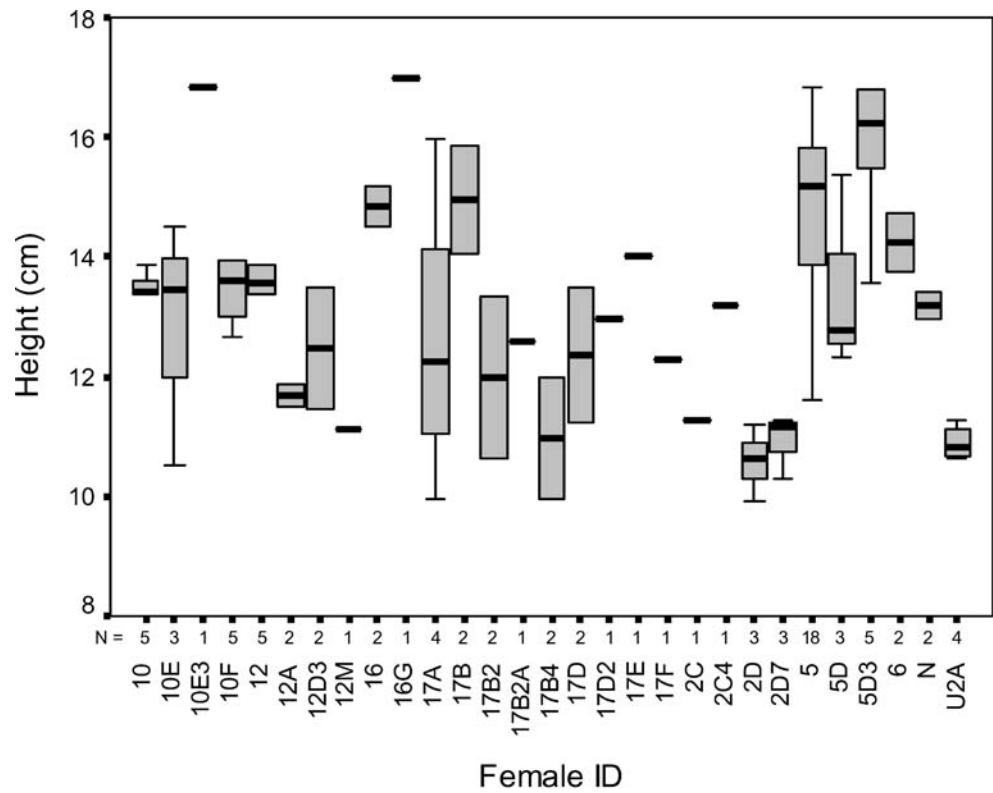
A total of 85 swelling cycles were measured for the 29 female subjects with 1–18 swelling cycles per female (mean±SEM 2.6±0.6). Female ID was a significant influence on swelling height ($F_{28, 55}=3.764$, $p_2 < 0.001$) and width ($F_{26, 53}=1.950$, $p_2=0.022$), meaning that variance between females was greater than the variance within females. However, swelling size did vary between cycles within females (Fig. 1). Swelling height varied by up to 9.7% between cycles for individual females ($n=20$ females contributing >1 cycle, mean 8.4±0.2%). Swelling width varied by up to 9.9% ($n=18$ females, mean 8.4±0.3%).

There was no significant difference in swelling height or width between a conceptive cycle and the previous non-conceptive cycle (paired tests, height: $n=14$ females, conceptive cycle 12.7±0.5, previous nonconceptive cycle 13.3±0.4, $t_{13}=1.70$, $p_2=0.113$; width: $n=12$ females, conceptive cycle 9.5±0.4, previous nonconceptive cycle 9.1±0.5, $t_{11}=0.76$, $p_2=0.466$). Two females each contributed two pairs of values to these tests, but selecting one of these pairs at random for the analyses did not alter the patterns observed. Conceptive and nonconceptive cycles were therefore combined for subsequent analyses.

Swelling size and parasites

Three taxa of amoebic protozoa (*Entamoeba coli*, *Endolimax nana*, and *Entamoeba histolytica/dispar* complex), one ciliate protozoa (*Balantidium coli*), and various nematodes were found in fecal samples collected from females during the study period. The abundance and percentage presence of *E. coli* and *E. nana*, percentage presence of *B. coli*, and mean parasite diversity over the annual cycle did not show sufficient interindividual variation to be informative in comparison with swelling size (the minimum value being more than 80% of the maximum, Table 1). Specific measures of parasitism (samples from the closest sampling month only) were also not sufficiently variable between females to allow for useful comparison with swelling size (Table 1). None of the other more variable measures of parasitism were significantly related to female sexual swelling size (Table 2). However, 18 of the 20 relationships tested were in the predicted negative direction, and statistical power in these tests was very low. For example,

Fig. 1 Variation in swelling height (*above*) and width (*below*) between individual females. The *box* represents the interquartile range. *Whiskers* indicate the highest and lowest values. *Lines* indicate the median value. Sample sizes appear below the *x*-axis



with a sample size of ten and a medium effect size ($r=0.3$), statistical power is only 0.232 (an analysis is judged powerful when power is >0.8 ; Cohen 1988). For a large effect size ($r=0.50$) statistical power is 0.510.

Swelling size and immune status

Swelling size was not significantly related to general measures of health, nor to health measures made at the

Table 1 Between-female variation in parasite measures

	General parasite measures ^a		Closest sampling month to swelling cycle ^b	
	Mean±SEM	Range	Mean±SEM	Range
Abundance				
Nematodes	1.8±0.1	1.0–2.4	1.4±0.2	1.0–3.0
<i>B. coli</i>	2.1±0.1	1.7–2.4	2.1±0.1	2.0–3.0
<i>E. histolytica/dispar</i>	1.5±0.1	1.1–2.0	1.6±0.3	0.0–2.0
<i>E. coli</i>	2.5±0.0	2.4–2.7	2.5±0.2	2.0–3.0
<i>E. nana</i>	2.7±0.1	2.5–3.0	2.9±0.1	2.0–3.0
Percentage presence				
Nematodes	78.7±6.1	37.5–100.0		
<i>B. coli</i>	96.5±1.9	83.3–100.0		
<i>E. histolytica/dispar</i>	80.3±4.2	62.5–100.0		
<i>E. coli</i>	100.0±0.0	100.0–100.0		
<i>E. nana</i>	100.0±0.0	100.0–100.0		
Parasite diversity	4.8±0.1	4.3–5.0	4.8±0.1	4.0–5.0

^a Females sampled in at least 6 of 12 sampling months only

^b Females contributing three samples only; percentage presence is not calculated for individual months

closest capture to each swelling (maximum delay 6 months, Table 3). The latter test involved multiple cycles from four females; choosing only one of these cycles at random for each female did not change the patterns observed (data not shown). Reducing the delay between capture and swelling cycle to 2 months reduced the sample size, but did not alter the significance of the results (Table 3).

All the observed relationships between swelling size and immune status were negative for swelling height, but positive for swelling width (Table 3). Statistical power was low for some of the tests. For example, with a sample size of 29 (matching that available for general measures and closest measure, maximum delay 6 months) and a medium effect size ($r=0.30$) power was 0.502. However, for a large effect size ($r=0.50$) power rose to 0.917. Thus, if there was a strong relationship between swelling size and general immunity, we would have been able to detect it. For a

sample size of 11 (matching closest measure, maximum delay 2 months), power was 0.248 ($r=0.3$) and 0.549 ($r=0.5$).

Swelling size and IR

IR varied from -0.29 to 0.24 in the female subjects (mean±SEM $-0.04±0.03$). Swelling height and width were not significantly related to IR (absolute height: $r=0.023$, $p_1=0.451$, $n=32$; absolute width: $r=0.019$, $p_1=0.460$, $n=30$; height corrected for standing height: $r=-0.197$, $p_1=0.140$, $n=32$; width corrected for standing height: $r=-0.241$, $p_1=0.100$, $n=30$). Statistical power in these tests was 0.514 for a medium effect size ($r=0.3$, based on a sample size of $n=30$) and 0.925 for a large effect size ($r=0.5$). Thus, if there was a strong relationship between swelling size and IR we would have been able to detect it.

Table 2 Results of Pearson's correlations comparing swelling size with general parasite measures, $n=10$

		Abundance			Percentage presence	
		Nematodes	<i>B. coli</i>	<i>E. histolytica /dispar</i>	Nematodes	<i>E. histolytica /dispar</i>
Absolute measures						
Height	r	0.140	-0.261	-0.084	-0.049	-0.047
	p_1 (Bonferroni threshold)	0.350 (0.010)	0.233 (0.006)	0.409 (0.017)	0.447 (0.025)	0.448 (0.050)
Width	r	-0.101	-0.154	-0.324	-0.250	-0.180
	p_1 (Bonferroni threshold)	0.391 (0.013)	0.335 (0.008)	0.181 (0.005)	0.243 (0.006)	0.309 (0.007)
Controlling for standing height						
Height	r	-0.051	-0.343	0.063	-0.520	0.227
	p_1 (Bonferroni threshold)	0.445 (0.025)	0.166 (0.006)	0.431 (0.017)	0.062 (0.005)	0.265 (0.010)
Width	r	-0.299	-0.095	-0.268	-0.277	-0.022
	p_1 (Bonferroni threshold)	0.201 (0.006)	0.397 (0.013)	0.228 (0.008)	0.219 (0.007)	0.477 (0.050)

Using general measures of parasitism over the year sampled; females sampled in at least 6 of 12 sampling months only
 p_1 =unadjusted one-tailed probability

Discussion

We found no significant relationships between sexual swelling size and female fitness, measured as parasitism, immune status, or genetic diversity. Although some of our statistical tests had low power, our results provide no support for the Hamilton and Zuk hypothesis, which predicts that individuals with low parasitism and high quality immune systems should show more exaggerated ornaments than those with high parasitism and poorer immune systems (Hamilton and Zuk 1982; Moller and Saino 1994). Nor do our results support Brown's (1997) hypothesis that exaggerated ornaments signal a high level of average heterozygosity.

Several caveats apply to our results concerning parasitism and health. First, there was surprisingly little interindividual variation in parasitism, by comparison with natural populations (reviews in Stuart and Strier 1995; Nunn and Altizer 2006). Some of the parasite taxa identified can be pathogenic in nonhuman primates and humans, and mediating the potentially damaging effects of parasite infection can be expected to be physiologically costly. However, the presence of intestinal parasites is well-tolerated in this provisioned population (Setchell et al., unpublished data) and it may be that we were only able to compare healthy animals, limiting the usefulness of this test. Second, costly sexual advertising is a life history trait that trades off with other components of reproduction and survival (Gustafsson et al. 1995; Hoglund and Sheldon 1998; Kokko et al. 2002). It is therefore difficult to draw conclusions from correlative tests of relationships between

condition (parasitism and immune status) and secondary sexual traits, as both positive and negative relationships can be explained as support for the Hamilton and Zuk hypothesis (Norris and Evans 2000; Kokko et al. 2002). Future work should use experimental techniques to examine the relationships between pathogen load, response to immune challenge, and ornamentation (Norris and Evans 2000; Cotton et al. 2004). This problem should not apply to our test of the relationship between swelling size and heterozygosity. However, it may be that even poor quality females receive sufficient resources in this provisioned colony to produce large swellings, confounding any relationships between swelling size and female genetic quality.

A crucial aspect of reliable indicator hypotheses is that members of the opposite sex should allocate more mating effort to individuals possessing larger ornaments (Zahavi 1975; Hamilton and Zuk 1982; Pagel 1994; Brown 1997). In support of this, swelling size increase within a cycle is associated with increased male mating effort in both mandrills (Setchell 1999) and other primates (reviewed in Nunn 1999). Experimental studies have also shown that captive male baboons masturbate more in response to supernormal-sized swellings (Bielert and Anderson 1985). Wild male baboons show greater interest in, and compete more intensely for, females with larger swellings (Domb and Pagel 2001). However, studies of both wild chimpanzees (Deschner et al. 2004) and the CIRMF mandrills (Setchell and Wickings 2004a) have failed to find a relationship between male mating effort and maximum swelling size.

Table 3 Results of Pearson's correlations comparing swelling size with the neutrophil/lymphocyte ratio

Swelling measure		General measure ^a	Closest measure, maximum delay 6 months ^b	Closest measure, maximum delay 2 months ^c
Absolute measures				
Height	<i>n</i>	31	34	14
	<i>r</i>	-0.007	-0.025	-0.179
	<i>p</i> ₁ (Bonferroni threshold)	0.486 (0.050)	0.445 (0.025)	0.271 (0.015)
Width	<i>n</i>	29	31	11
	<i>r</i>	0.167	0.154	0.207
	<i>p</i> ₁ (Bonferroni threshold)	0.193 (0.008)	0.205 (0.010)	0.271 (0.015)
Controlling for standing height				
Height	<i>n</i>	31	35	14
	<i>r</i>	-0.100	-0.220	-0.410
	<i>p</i> ₁ (Bonferroni threshold)	0.296 (0.025)	0.102 (0.010)	0.073 (0.008)
Width	<i>n</i>	29	34	13
	<i>r</i>	0.123	0.049	0.330
	<i>p</i> ₁ (Bonferroni threshold)	0.263 (0.017)	0.393 (0.050)	0.135 (0.013)

^a General immune measure using the mean of all values available for each individual

^b Specific immune measure, mean delay from capture to swelling 2.6±0.3 months, includes multiple contributions from four females

^c Specific immune measure, mean delay from capture to swelling 1.3±0.1 months, each female contributed a single swelling cycle
*p*₁=unadjusted one-tailed probability

Between-female variation in swelling size was greater than within-female variation in these mandrills, indicating that swelling characteristics were enduring features of individual females. However, measures from multiple cycles of individual females also showed variation. This supports the hypothesis that swelling size may indicate cycle-to-cycle variability in the probability that an individual female conceives (Zinner et al. 2002; Emery and Whitten 2003). However, no significant size differences between conceptive and nonconceptive swellings were observed. This contrasts with wild chimpanzees where both swelling size and mean estrogen titer during the periovulatory phase increase in consecutive cycles and are highest in conceptive cycles (Deschner et al. 2004). Domb and Pagel (2001) have also reported no difference between the conceptive and the closest nonconceptive cycle in baboons. However, this result is based on a small number of conceptive cycles and a trend toward larger swellings during conceptive cycles did occur (Zinner et al. 2002; Emery and Whitten 2003). The consistency of swelling size between conceptive and nonconceptive cycles in the CIRMF mandrills may be due to provisioning in the mandrill colony, which may artificially equalize differences in female fecundity. In contrast with wild baboons where females undergo multiple cycles before conception (Altmann et al. 1977; Bentley-Condit and Smith 1997; Weingrill et al. 2003), the mandrills generally conceive within two swelling cycles (Setchell and Wickings 2004b). This limits our ability to examine subsequent cycles. Future studies should include hormonal data to test the fertility of nonconceptive cycles.

In conclusion, potential differences in the demography and feeding ecology of this semicaptive population, compared with wild mandrills, limit the interpretation of evolutionary patterns. However, in combination with previous findings that sexual swelling size is unrelated to female reproductive history, and that males do not preferentially allocate mating effort to females possessing larger swellings (Setchell and Wickings 2004a), our results suggest that swelling size does not act as a reliable indicator of female quality in these mandrills. Swelling size does appear to vary from cycle to cycle within females, allowing for the possibility that swelling size indicates individual cycle quality (Zinner et al. 2002; Emery and Whitten 2003). The necessary hormonal data to test this hypothesis are currently lacking, although it seems likely that the majority of cycles are of high quality in these provisioned animals.

Acknowledgements We wish to thank CIRMF for making the study possible. We particularly thank the past and present staff of the Primate Centre and the LAM at CIRMF. We thank Benoît Goossens and Aurelie Gauthier for help with fecal sampling, and Charlie Nunn and three anonymous reviewers for comments that greatly improved the manuscript. The CIRMF is financed by the Gabonese government,

Total Gabon, and the Ministère Français des Affaires Etrangères. This study was funded by a Leverhulme Trust project grant (award no. F/01576/B).

References

- Abernethy KA, White LJT, Wickings EJ (2002) Hordes of mandrills (*Mandrillus sphinx*): extreme group size and seasonal male presence. *J Zool* 258:131–137
- Able DJ (1996) The contagion indicator hypothesis for parasite-mediated sexual selection. *Proc Natl Acad Sci U S A* 93:2229–2233
- Alberts SC, Altmann J, Wilson ML (1996) Mate guarding constrains foraging activity of male baboons. *Anim Behav* 51:1269–1277
- Altmann SA (1962) A field study of the sociobiology of the rhesus monkey, *Macaca mulatta*. *Ann NY Acad Sci* 102:338–435
- Altmann J, Altmann SA, Hausfater G, McCuskey S (1977) Life history of yellow baboons: physical development, reproductive parameters and infant mortality. *Primates* 18:315–330
- Amos W, Worthington Wilmer J, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T (2001) The influence of parental relatedness on reproductive success. *Proc R Soc Lond Ser B Biol Sci* 268:2021–2027
- Amundsen T (2000) Why are female birds ornamented? *Trends Ecol Evol* 15:149–155
- Andersson M (1994) Sexual selection. Princeton University Press, Princeton, New Jersey
- Aparicio JM, Cordero PJ, Veiga JP (2001) A test of the hypothesis of mate choice based on heterozygosity in the spotless starling. *Anim Behav* 62:1001–1006
- Bentley-Condit VK, Smith EO (1997) Female reproductive parameters of Tana River yellow baboons. *Int J Primatol* 16:581–596
- Bercovitch FB (1983) Time budgets and consortships in olive baboons (*Papio anubis*). *Folia Primatol* 41:180–190
- Bielert C, Anderson CM (1985) Baboon sexual swellings and male response: a possible operational mammalian supernormal stimulus and response interaction. *Int J Primatol* 6:377–393
- Bielert C, Girolami C (1986) Experimental assessments of behavioural and anatomical components of female chacma baboon (*Papio ursinus*) sexual attractiveness. *Psychoneuroendocrinology* 11:75–90
- Bielert C, van der Walt LA (1982) Male chacma baboon (*Papio ursinus*) sexual arousal: mediation by visual cues from female conspecifics. *Psychoneuroendocrinology* 7:31–48
- Bielert C, Girolami L, Jowell S (1989) An experimental examination of the colour component in visually mediated sexual arousal of the chacma baboons (*Papio ursinus*). *J Zool* 219:569–579
- Brown JL (1997) A theory of mate choice based on heterozygosity. *Behav Ecol* 8:60–65
- Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Ann Rev Ecol Syst* 18:237–268
- Charpentier M, Setchell JM, Prugnolle F, Knapp LA, Wickings EJ, Peignot P, Hossaert-McKey M (2005) Genetic diversity and reproductive success in mandrills (*Mandrillus sphinx*). *Proc Natl Acad Sci U S A* 102:16723–16728
- Cohen J (1988) Statistical power analysis for the behavioral sciences. Lawrence Erlbaum, New Jersey
- Cotton S, Fowler K, Pomiankowski A (2004) Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc Biol Sci* 271:771–783
- Deschner T, Heistermann M, Hodges K, Boesch C (2004) Female sexual swelling size, timing of ovulation, and male behavior in wild West African chimpanzees. *Horm Behav* 46:204–215

- Dewsbury DA (1982) Ejaculate cost and male choice. *Am Nat* 119:601–610
- Ditchkoff SS, Lochmiller RL, Masters RE, Hooper ST, van den Bussche RA (2001) Major-histocompatibility-complex-associated variation in secondary sexual traits of white-tailed deer (*Odocoileus virginianus*): evidence for good-genes advertisement. *Evolution* 55:616–625
- Dixson AF (1983) Observations on the evolution and behavioural significance of 'sexual skin' in female primates. *Adv Study Behav* 13:63–106
- Dixson AF (1998) Primate sexuality: comparative studies of the prosimians, monkeys, apes and human beings. Oxford University Press, Oxford
- Domb LG, Pagel M (2001) Sexual swellings advertise female quality in wild baboons. *Nature* 410:204–206
- Domb LG, Pagel M (2002) Reply: significance of primate sexual swellings. *Nature* 420:143
- Draws C (1996) Contexts and patterns of injuries in free-ranging male baboons (*Papio cynocephalus*). *Behaviour* 133:443–474
- Edwards SV, Hedrick PW (1998) Evolution and ecology of MHC molecules: from genomics to sexual selection. *Trends Ecol Evol* 13:305–311
- Emery MA, Whitten PL (2003) Size of sexual swellings reflects ovarian function in chimpanzees (*Pan troglodytes*). *Behav Ecol Sociobiol* 54:340–351
- Erdfelder E, Faul F, Buchner A (1996) GPOWER: a general power analysis program. *Behav Res Meth Instrum Comput* 28:1–11
- Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature* 425:714–717
- Grubb P (1973) Distribution, divergence and speciation of the drill and mandrill. *Folia Primatol* 20:161–177
- Gustafsson L, Qvanstroem A, Sheldon B (1995) Trade-offs between life history traits and a secondary sexual character in male collared flycatchers. *Nature* 375:311–313
- Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites. *Science* 218:384–387
- Harrison MJS (1988) The mandrills in Gabon's rain forest: ecology, distribution and status. *Oryx* 22:218–228
- Hoffman JI, Boyd IL, Amos W (2004) Exploring the relationship between parental relatedness and male reproductive success in the Antarctic fur seal *Arctocephalus gazella*. *Evolution* 58:2087–2099
- Hoglund J, Sheldon BC (1998) The cost of reproduction and sexual selection. *Oikos* 83:478–483
- Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. *Biol Rev* 75:21–64
- Johnstone RA (1995) Sexual selection, honest advertisement and the handicap principle—reviewing the evidence. *Biol Rev* 70:1–65
- Keller L, Waller DM (2002) Inbreeding effects in wild populations. *Trends Ecol Evol* 17:230–241
- Kim C-Y, Lee H-S, Han S-C, Heo J-D, Kwon M-S, Ha C-S, Han S-S (2005) Hematological and serum biochemical values in cynomolgus monkeys anesthetized with ketamine hydrochloride. *J Med Primatol* 34:96–100
- Kokko H, Brooks R, McNamara JM, Houston AI (2002) The sexual selection continuum. *Proc R Soc Lond B Biol Sci* 269:1331–1340
- Marshall RC, Buchanan KL, Catchpole CK (2003) Sexual selection and individual genetic diversity in a songbird. *Proc R Soc Lond B Biol Sci* 270:S248–S250
- Marson J, Gervais D, Meuris S, Cooper RW, Jouanet P (1989) Influence of ejaculation frequency on semen characteristics in chimpanzees (*Pan troglodytes*). *J Reprod Fertil* 85:43–45
- Mitton JB, Schuster WSF, Cothran EG, de Fries JC (1993) Correlation between the individual heterozygosity of parents and their offspring. *Heredity* 71:59–63
- Møller AP, Saino N (1994) Parasites, immunology of hosts, and host sexual selection. *J Parasitol* 80:850–858
- Møller AP, Christe P, Lux E (1999) Parasitism, host immune function, and sexual selection. *Q Rev Biol* 74:3–20
- Müller G, Ward PI (1995) Parasitism and heterozygosity influence the secondary sexual characters of the European minnow, *Phoxinus phoxinus* (L) (Cyprinidae). *Ethology* 100:309–319
- Norris K, Evans MR (2000) Ecological immunology: life history trade-offs and immune defence in birds. *Behav Ecol* 11:19–26
- Nunn CL (1999) The evolution of exaggerated sexual swellings in primates and the graded signal hypothesis. *Anim Behav* 58:299–246
- Nunn CL, Altizer S (2006) Infectious diseases in primates: behavior, ecology and evolution. Oxford University Press, Oxford
- Nunn CL, van Schaik CP, Zinner D (2001) Do exaggerated sexual swellings function in female mating competition in primates? A comparative test of the reliable indicator hypothesis. *Behav Ecol* 12:646–654
- Pagel M (1994) The evolution of conspicuous oestrous advertisement in old world monkeys. *Anim Behav* 47:1333–1341
- Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. *Am Nat* 153:145–164
- Preston BT, Stevenson IR, Pemberton JM, Wilson K (2001) Dominant rams lose out by sperm depletion: a waning success in siring counters a ram's high score in competition for ewes. *Nature* 409:681–682
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Scribner KT, Smith MH, Johns PE (1989) Environmental and genetic components of antler growth in white-tailed deer. *J Mammal* 70:284–291
- Setchell JM (1999) Socio-sexual development in the male mandrill (*Mandrillus sphinx*). University of Cambridge, Cambridge, UK
- Setchell JM, Wickings EJ (2004a) Sexual swellings in mandrills (*Mandrillus sphinx*): a test of the reliable indicator hypothesis. *Behav Ecol* 15:438–445
- Setchell JM, Wickings EJ (2004b) Social and seasonal influences on the reproductive cycle in female mandrills (*Mandrillus sphinx*). *Am J Phys Anthropol* 125:73–84
- Setchell JM, Tshipamba P, Bourry O, Rouquet P, Wickings EJ, Knapp LA (2006) Haematology of a semi-free-ranging colony of mandrills (*Mandrillus sphinx*). *Int J Primatol* (in press)
- Shaikh AA, Celaya CL, Gomez I, Schaik SA (1982) Temporal relationship of hormonal peaks to ovulation and sex skin deturgescence in the baboon. *Primates* 23:444–452
- Sheridan L, Pomiankowski A (1997) Fluctuating asymmetry, spot asymmetry and inbreeding depression in the sexual coloration of male guppy fish. *Heredity* 79:515–523
- Snowdon CT (2004) Sexual selection and communication. In: Kappeler PK, van Schaik CP (eds) *Sexual selection in primates: new and comparative perspectives*. Cambridge University Press, Cambridge, pp 57–70
- Stallmann RR, Froehlich JW (2000) Primate sexual swellings as coevolved signal systems. *Primates* 41:1–16
- Stuart MD, Strier KB (1995) Primates and parasites: a case for a multidisciplinary approach. *Int J Primatol* 16:577–593
- van Oosterhout C, Trigg RE, Carvalho GR, Magurran AE, Hauser L, Shaw PW (2003) Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. *J Evol Biol* 16:273–281
- von Schantz T, Wittzell H, Goransson G, Grahm M, Persson K (1996) MHC genotype and male ornamentation: genetic evidence for the Hamilton–Zuk model. *Proc R Soc Lond Ser B Biol Sci* 263:265–271
- von Schantz T, Wittzell H, Goransson G, Grahm M (1997) Mate choice, male condition-dependent ornamentation and MHC in the pheasant. *Heredity* 127:133–140

- Wedell N, Gage MJG, Parker GA (2002) Sperm competition, male prudence and sperm-limited females. *Trends Ecol Evol* 17:313–320
- Weingrill T, Lycett JE, Barrett L, Hill RA, Henzi SP (2003) Male consortship behaviour in chacma baboons: the role of demographic factors and female conceptive probabilities. *Behaviour* 140:405–427
- Wildt DE, Doyle U, Stone SC, Harrison RM (1977) Correlation of perineal swelling with serum ovarian hormone levels, vaginal cytology and ovarian follicular development during the baboon reproductive cycle. *Primates* 18:261–270
- Zahavi A (1975) Mate selection—a selection for handicap. *J Theor Biol* 53:205–214
- Zinner D, Alberts SC, Nunn CL, Altmann J (2002) Significance of primate sexual swellings. *Nature* 420:142–143
- Zuk M (1992) The role of parasites in sexual selection: current evidence and future directions. *Adv Study Behav* 21:39–68