



HAL
open science

Inbreeding depression in non-human primates: a historical review of methods used and empirical data

Marie Charpentier, Anja Widdig, Susan C. Alberts

► To cite this version:

Marie Charpentier, Anja Widdig, Susan C. Alberts. Inbreeding depression in non-human primates: a historical review of methods used and empirical data. *American Journal of Primatology*, 2007, 69 (12), pp.1370-1386. 10.1002/ajp.20445 . hal-02079454

HAL Id: hal-02079454

<https://hal.science/hal-02079454v1>

Submitted on 9 Dec 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RESEARCH ARTICLE

Inbreeding Depression in Non-Human Primates: A Historical Review of Methods Used and Empirical Data

M.J.E. CHARPENTIER^{1,2*}, A. WIDDIG^{1,3,4}, AND S.C. ALBERTS^{1,5}

¹*Department of Biology, Duke University, Durham, North Carolina*

²*CEFE-CNRS UMR 5175, Montpellier Cedex 5, France*

³*Department of Primatology, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany*

⁴*Caribbean Primate Research Center, University of Puerto Rico, Punta Santiago, Puerto Rico*

⁵*Institute of Primate Research, National Museums of Kenya, Nairobi, Kenya*

Offspring born to related parents may show reduced fitness due to inbreeding depression. Although evidence of inbreeding depression has accumulated for a variety of taxa during the past two decades, such analyses remain rare for primate species, probably because of their long generation time. However, inbreeding can have important fitness costs and is likely to shape life-history traits in all living species. As a consequence, selection should have favored inbreeding avoidance via sex-biased dispersal, extra-group paternity, or kin discrimination. In this paper, we review empirical studies on the effects of inbreeding on fitness traits or fitness correlates in primate species. In addition, we report the methods that have been used to detect inbreeding in primate populations, and their development with the improvement of laboratory techniques. We focus particularly on the advantages and disadvantages using microsatellite loci to detect inbreeding. Although the genetic data that are typically available (partial pedigrees, use of microsatellite heterozygosity as an estimate of genomewide inbreeding) tend to impose constraints on analyses, we encourage primatologists to explore the potential effects of inbreeding if they have access to even partial pedigrees or genetic information. Such studies are important because of both the value of basic research in inbreeding depression in the wild and the conservation issues associated with inbreeding, particularly in threatened species, which include more than half of the currently living primate species. *Am. J. Primatol.* 69:1370–1386, 2007. © 2007 Wiley-Liss, Inc.

Contract grant sponsor: Emmy Noether; Contract grant number: WI 1801/1-1 and 2-1; Contract grant sponsor: National Science Foundation; Contract grant number: IOB-0322613 and BCS-0323553; Contract grant sponsor: National Center for Research Resources (NCRR); Contract grant number: CM-5 P40 RR003640-20.

*Correspondence to: Dr M.J.E. Charpentier, Department of Biology, Duke University, Box 90338, Duke University, Durham, NC 27708. E-mail: mariecharp@yahoo.fr

Key words: Inbreeding, inbred mating, primates, genetic analysis, pedigrees, heterozygosity

INTRODUCTION

Inbreeding, which occurs in offspring born to related parents, has well-defined genetic consequences, i.e., an increase of homozygosity in the whole genome [Charlesworth & Charlesworth, 1999]. The consequences of increased homozygosity at loci affecting fitness are often negative, either because recessive deleterious alleles are expressed or because homozygosity reduces heterozygote advantage [Charlesworth & Charlesworth, 1987]. The strong detrimental effects of inbreeding in animal populations can include reduced overall fertility [Amos et al., 2001; Charpentier et al., 2005a; Gallardo et al., 2004; Hoffman et al., 2004; Seddon et al., 2004; Slate et al., 2000], affected growth [Charpentier et al., 2006; Coltman et al., 1998; Pujolar et al., 2005], reduced survival [Coulson et al., 1998, 1999; Mandal et al., 2004], and increased disease susceptibility [Acevedo-Whitehouse et al., 2003]. Inbreeding depression thus appears to have the potential to shape life history, behavioral, morphological and physiological traits in all living species, from simple to complex. Selection to avoid inbreeding has been proposed as an important force influencing a range of behavioral traits. For instance, inbreeding avoidance is suggested to be one of the ultimate causes of sex-biased dispersal or extra-group copulations [Pusey, 1990; Pusey & Packer, 1987; Pusey & Wolf, 1996; see however Perrin & Mazalov, 1999; 2000]. Inbreeding avoidance may also shape social relationships, such as mate choice behaviors: individuals generally avoid mating with relatives whom they encounter as potential mates, which also implies the ability to discriminate kin [Pusey & Wolf, 1996]. Reproductive suppression and delayed reproduction have also been proposed as potential inbreeding avoidance mechanisms [Blouin & Blouin, 1988]. The evolution of inbreeding avoidance behaviors will depend on their plasticity to respond to the costs associated with inbreeding depression.

Population genetic models predict that the increased homozygosity resulting from inbreeding will expose recessive deleterious alleles to selection and, as a consequence, further inbreeding will become harmless, after purging of the genetic load [Waller, 1993]. Indeed, some studies find no inbreeding depression in their studied species, suggesting the possibility that such purging may have occurred [Peer & Taborskyi, 2005; Thünken et al., 2007]. These and similar findings have led some researchers to question whether inbreeding depression really occurs in natural populations [see for review: Keller & Waller, 2002]. Nevertheless, even if lethal and semi-lethal mutations can be quickly purged via inbreeding, the large fitness costs of this purging may affect population viability [Barrett & Charlesworth, 1991]. Further, mutations that are only mildly deleterious are difficult to eliminate and are the principle cause of inbreeding depression [van Oosterhout et al., 2000; Wang, 2000; Willis, 2000]. Moreover, many animal species experience gene flow at high enough rates to reintroduce genetic load quickly via immigrants. The theoretical and experimental results reviewed in Keller and Waller [2002] suggest that purging will not substantially reduce inbreeding depression for most wild populations. Studies on inbreeding depression are thus highly pertinent, particularly in threatened populations facing habitat destruction and fragmentation.

Small, fragmented populations, with limited sex-biased dispersal, perhaps due to deforestation or other anthropogenic disturbance, will experience population subdivision and hence potentially severe inbreeding depression.

Populations with a reduced number of individuals and limited or fragmented distribution often show lower genetic variability compared with widely distributed species in large continuous habitats because of the higher probability of the occurrence of genetic drift and inbreeding [Frankham, 2003; Goossens et al., 2006]. Results from Goossens et al. [2006] strongly suggested that recent anthropogenic environmental changes were the main cause of the orang-utan (*Pongo pygmaeus*) population collapse in Sabah (northern Borneo). They also found that this decline had already led to the loss of significant genetic diversity, mostly through the loss of rare alleles. Inbreeding depression has been demonstrated to cause extinctions in deliberately inbred populations [Frankham, 1995, 2003; Newman & Pilson, 1997], and has also contributed to extinction in one natural population of butterflies [Saccheri et al., 1998]. Further, populations with low genetic diversity are expected to suffer more seriously from diseases, pests, and parasites than those with high genetic diversity [Frankham et al., 2002]. Currently, much information on inbreeding and extinctions come from species used in laboratory experiments. It is therefore essential to know whether these findings can be extrapolated to other species and taxonomic groups.

Although the incidences and consequences of inbreeding have been well studied during the last two decades in a variety of taxa [Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999; Keller & Waller, 2002], such analyses are rare for primates, probably because of their long generation time. Studying inbreeding depression requires comprehensive data on life-history traits, which are not always easily retrievable, especially in species living in closed environment such as tropical forests, the habitat of about 90% of primate species. It also requires genetic analyses or detailed and deep pedigrees for the detection of inbred individuals. Another notable problem for estimating the severity of inbreeding depression in free-ranging or wild populations through the use of genetic markers is that an important fraction of neonates die each year in both captive and wild primate populations before paternity can be assigned (14.4% of newborns died before 1 year of age in rhesus macaques from Cayo Santiago [A. Widdig, unpublished data] and 35% of conceptions resulted in either fetal losses or deaths in the first year of life in wild savanna baboons from Amboseli [Altmann & Alberts, 2003; Beehner et al., 2006a,b]). We do not know yet if fetal losses or individuals that die as young infants tend to be more inbred than those that survive, but, if so, population rates of inbreeding are likely to be underestimated.

In this review, we present the empirical studies to date on the effects of inbreeding on fitness traits in primates. Second, we review and discuss the methods used to detect inbreeding in primate populations and their development with the improvement of laboratory techniques. We focus particularly on the advantages and disadvantages of the use of microsatellite heterozygosity as an estimate of genome-wide inbreeding. Because inbreeding depression constitutes an important threat that can directly affect population persistence, and because 161 primate species are facing high risks of extinction in their natural occupancy (of 274 evaluated, i.e., 59%; IUCN 2006 red list), the aim of this review is to encourage primatologists to explore the potential effects of inbreeding in their studied populations if they possess even partial pedigrees or genetic information.

EMPIRICAL DATA ON INBREEDING DEPRESSION IN PRIMATES

Studies of captive-bred primates have often cited inbreeding depression as the cause of a wide range of congenital malformations. These include fused labia

in common marmoset (*Callithrix jacchus*) with affected females being functionally infertile [Isachenko et al., 2002]; anencephalic and acranial females and blind males in rhesus macaques (*Macaca mulatta*; Rawlins & Kessler [1983]); limb malformations in rhesus and Japanese macaques (*Macaca fuscata*; Nakamichi et al. [1997]); defects of the diaphragm in golden lion tamarins (*Leontopithecus rosalia*; Bush et al. [1980]); a supernumerary left kidney in squirrel monkeys (*Saimiri sciureus*; Stills & Bullock [1981]); achondroplastic-like dwarfism, polydactylism and syndactylism in neonatal tamarins (*Saguinus oedipus* and *Saguinus fuscicollis*; Chalifoux & Elliott [1986]); and greater risk of contracting human lymphoma in hamadryas baboons [*Papio hamadryas*; Crawford & O'Rourke, 1978; but see Crawford et al., 1984, 1987].

However, although these studies cited inbreeding depression as the likely cause of these congenital malformations, they did not quantify inbreeding. Researchers began to attempt to quantify inbreeding and measure its fitness effects in primate populations in the late 1970s. Among the first was a study by Ralls and Ballou [1982], which involved an analysis of inbreeding depression acting on infant survival in 16 captive primate species, using breeding records. They found that mortality was higher for inbred infant in 15 out of 16 studied species. We have identified 14 other studies on wild, free-ranging, or captive populations between 1979 and 2006, and have summarized these 14 studies in Table I (those in which inbred individuals experienced a loss of fitness) and Table II (those that found no effect of inbreeding). The results summarized in Tables I and II illustrate several important points about inbreeding depression. First, they show that inbreeding may not affect all fitness traits equally in different populations of the same species or even in the same population. Indeed, the intensity of inbreeding depression may vary depending on life-history stage, trait measured, experimental habitat, or environmental conditions [Keller & Waller, 2002]. Such variation in susceptibility to inbreeding should reflect the interaction of genes with the environment and may also reflect the fact that some traits are influenced by more or fewer loci showing more or less directional dominance [Keller & Waller, 2002]. For example, in captive rhesus macaques, although inbred offspring displayed lower (estimated) birth weight, inbreeding depression did not occur for infant growth, yearling mortality, morbidity, or female fertility [Smith, 1986]. In semi-free-ranging mandrills (*Mandrillus sphinx*), reproductive success of both females and alpha males were affected by inbreeding depression—i.e., females and alpha males that were more inbred experienced reduced reproduction relative to those that were not inbred. In contrast, the reproductive success of subordinate males was not affected by inbreeding depression—i.e., inbred and non-inbred subordinates reproduced equally well [Charpentier et al., 2005a].

Second, sensitivity to inbreeding has been related to gender which is probably a result of different physiological trajectories of development as well as different life-history traits between the sexes [Charpentier et al., 2006, see also in non-primate species: Coulson et al., 1999; Prugnolle et al., 2004]. Inbreeding depression influenced female growth (both height and weight), but not male growth in mandrills [Charpentier et al., 2006 and see discussion on the potential causes therein]. Lacy et al. [1993] also showed sex differences in survival in captive *Callimico*. Inbreeding depression in male offspring was much weaker than in female offspring: only males sired by full-sib and parent-offspring matings ($f = 0.25$) had lower survival. In *Callimico*, inbreeding depression was much more severe in females than in males and even females sired by first cousins or less related animals ($f = 0.0625$) showed lower survival than non-inbred females.

TABLE I. Deleterious Effects of Inbreeding Depression Recorded in Primate Species

Species	Condition	Method used to detect inbreeding	Parameters analyzed	Inbreeding effects	Sample size	Inbreeding coefficient (f) or heterozygosity (Hs) range	Source
<i>Papio anubis</i>	Wild	Behavioral observations of matings	One-month survival	Potentially inbred conceptions resulted in 50% of offspring mortality before 1 month of age while potentially non-inbred conceptions resulted in 15.8% of mortality	$N_1 = 8$; $N_2 = 38$	Unknown	Packer [1979]
<i>Macaca mulatta</i>	Captive groups	Paternity analysis using protein electrophoresis (28 markers)	Birth weight	Lower birth weight (estimated) in inbred offspring compared to non-inbred offspring	$N_1 = 101$; $N_2 = 94$	Unknown	Smith [1986]
<i>Varecia variegata variegata</i>	Captive groups	Maternal inbreeding coefficient retrieved from Studbook	Survival to 5 days	Inbred offspring died at higher rates than non-inbred offspring	$N_1 = 124$; $N_2 = 171$	$f = 0-0.358$	Noble et al. [1990]
<i>Callimico goeldii</i>	Captive groups	Pedigree recorded from monogamous pairs	Survival to 1-month	Inbred conceptions resulted in 46% offspring death before 1 month of age while non-inbred conceptions resulted in 24% mortality	$N_1 = 111$; $N_2 = 679$	$f = 0-0.25$	Lacy et al. [1993]
<i>Leontopithecus rosalia</i>	Wild	Behavioral observations of matings	Infant survival	Potentially inbred conceptions resulted in 100% offspring death before weaning while potentially non-	$N_1 = 14$ litters; $N_2 = 5$ litters	$f = 0-0.25$	Dietz and Baker [1993]

<i>Papio cynocephalus</i>	Wild	Behavioral observations of consortships	Survival to 1-month	inbred conceptions resulted in 86% infant survival (at least one offspring per litter) Potentially inbred conceptions (with natal males) resulted in 100% offspring death before 30 days while potentially non-inbred conceptions resulted in 19% infant mortality	$N_1 = 3$; $N_2 = 140$	Unknown	Alberts and Altmann [1995]
<i>Pan troglodytes schweinfurthii</i>	Wild	Paternity analysis using microsatellite loci and pedigree	Survival	The conception between a mother-son dyad resulted in an offspring that died during infancy	$N_1 = 1$; $N_2 = 13$	$f = 0-0.25$	Constable et al. [2001]
<i>Cercopithecus solatus</i>	Semi-free ranging	Paternity analysis using microsatellite loci and pedigree	Inter-birth intervals in mothers	Longer inter-birth intervals following births of inbred offspring vs. non-inbred offspring	$N_1 = 3$; $N_2 = 6$	$f = 0-0.25$	Charpentier et al. [2005b]
<i>Mandrillus sphinx</i>	Semi-free ranging	Paternity analysis using microsatellite loci and estimates of heterozygosity	Reproductive success and male dominance	1. More heterozygous females produce more offspring than less heterozygous ones, 2. more heterozygous alpha males sire more offspring, and, 3. more heterozygous α -males show longer tenure than more homozygous α -males	1. $N = 52$ 2. $N = 9$ 3. $N = 12$	1. $H_s = 0-1$ 2. $H_s = 0.75-1$ 3. $H_s = 0-1$	Charpentier et al. [2005a]

TABLE I. Continued

Species	Condition	Method used to detect inbreeding	Parameters analyzed	Inbreeding effects	Sample size	Inbreeding coefficient (f) or heterozygosity (Hs) range	Source
<i>Mandrilus sphinx</i>	Semi-free ranging	Paternity analysis using microsatellite loci and pedigree	Morphological parameters, reproductive success	Inbred females: 1. are lighter, 2. are smaller, and, 3. give birth earlier	1. $N_1 = 30$; $N_2 = 64$ 2. $N_1 = 26$; $N_2 = 58$ 3. $N_1 = 20$; $N_2 = 49$	1. $f = 0-0.25$ 2. $f = 0-0.25$ 3. $f = 0-0.25$	Charpentier et al. [2006]

Studies are classified chronologically.

N : total sample size when considering genetic diversity.

N_1 : inbred individuals or estimated inbred individuals (data obtained from a pedigree).

N_2 : non-inbred individuals or estimated non-inbred individuals (data obtained from a pedigree).

TABLE II. No Effect of Inbreeding Depression Detected in Primate Species, on Various Fitness Traits or Correlates

Species	Condition	Method used to detect inbreeding	Parameters analyzed	Sample size and genetic data	f or heterozygosity (H) range	Source
<i>Papio hamadryas</i>	Captive groups	Pedigree and paternity analysis using protein electrophoresis	Lymphoma disease	$N_1 = 173$; $N_2 = 383$	Unknown	Crawford et al. [1984, 1987]
<i>Macaca mulatta</i>	Captive groups	Paternity analysis using protein electrophoresis	<ol style="list-style-type: none"> 1. Infant growth rate, 2. female fertility, 3. mortality before 1 year of age, 4. morbidity 	<ol style="list-style-type: none"> 1. $N_1 = 101$; $N_2 = 94$ 2. $N_1 = 7$; $N_2 = 7$ 3. $N_1 = 40$; $N_2 = 40$ 4. $N_1 = 36$; $N_2 = 30$ 	Unknown	Smith [1986]
<i>Papio c. ursinus</i>	Wild	Behavioral observations of consortships	<p>Infant survival to 30 and 90 days following potentially inbred vs. non-inbred consortships</p> <p>Survival to 5 days</p>	$N_1 = 20$; $N_2 = 27$	Unknown	Bulger and Hamilton [1988]
<i>Varecia variegata rubra</i>	Captive groups	Maternal inbreeding coefficient retrieved from Studbook	Survival to 5 days	$N_1 = 48$; $N_2 = 30$	$f = 0-0.25$	Noble et al. [1990]
<i>Macaca mulatta</i>	Captive groups	Paternity analysis using protein electrophoresis	<ol style="list-style-type: none"> 1. Growth rate, 2. reproductive success in females, 	<ol style="list-style-type: none"> 1. $N_1 = 89$; $N_2 = 140$ 2. $N_1 = 56$; $N_2 = 73$ 	<ol style="list-style-type: none"> 1. $f = 0-0.25$ 2. $f = 0-0.25$ 3. $f = 0-0.25$ 	Smith [1995]

TABLE II. Continued

Species	Condition	Method used to detect inbreeding	Parameters analyzed	Sample size and genetic data	f or heterozygosity (H) range	Source
<i>Mandrillus sphinx</i>	Semi-free ranging	Paternity analysis using microsatellite loci and estimates of heterozygosity	3. mortality before 2 years of age	3. $N_1 = 91$; $N_2 = 141$		
			1. Subordinate male reproductive success, 2. female dominance, and, 3. acquisition of α status in males	1. $N = 39$ 2. $N = 72$ 3. $N = 59$	1. $H_s = 0.5-1$ 2. $H_s = 0-1$ 3. $H_s = 0.5-1$	Charpentier et al. [2005a]
<i>Mandrillus sphinx</i>	Semi-free ranging	Paternity analysis using microsatellite loci and pedigree	1. Longevity in both sexes, 2. weight in males, and, 3. height in males	1. $N_1 = 58$; $N_2 = 135$ 2. $N_1 = 27$; $N_2 = 70$ 3. $N_1 = 22$; $N_2 = 67$	1. $f = 0-0.25$ 2. $f = 0-0.25$ 3. $f = 0-0.25$	Charpentier et al. [2006]

Studies are classified chronologically.

N : total sample size when considering genetic diversity.

N_1 : inbred individuals or estimated inbred individuals (data obtained from a pedigree).

N_2 : non-inbred individuals or estimated non-inbred individuals (data obtained from a pedigree).

Because mortality among non-inbred offspring was similar between males and females, the authors suggested that “genetically debilitated females are more likely to die” and that the “investigations of parental care might reveal interesting sex biases” [Lacy et al., 1993].

Tables I and II are ordered chronologically, revealing both the evolution of the techniques used to assess inbreeding, and a change in the fitness traits studied to assess inbreeding depression. The development of new molecular techniques (e.g., DNA fingerprinting, PCR-based microsatellite genotyping, and automated DNA sequencing) has allowed the progressive replacement of behavioral observations by genetic paternity determination. Furthermore, although early studies concentrated on infant survival [e.g., Ralls & Ballou, 1982], a diverse set of life history and behavioral traits have now been analyzed in some species (reproductive success, dominance, morphological parameters, etc.). It may be that the development of molecular techniques has been paralleled by an increase in both the quantity (number of generations available) and quality (diversification of traits analyzed) of our knowledge of primates.

METHODS USED TO DETECT INBREEDING AND INBREEDING DEPRESSION: STRENGTHS AND WEAKNESSES

Behavioral Observations of Potentially Inbred vs. Non-Inbred Reproductions and Potential Errors

The first studies on inbreeding depression in primates concerned mainly wild populations, primarily of macaques and baboons [but also see Ralls & Ballou, 1982]. Behavioral observations of sexual activities between reproductive individuals were used to determine whether conceptions involved relatives, generating inbred offspring [Alberts & Altmann, 1995; Bulger & Hamilton, 1988; Dietz & Baker, 1993; Packer, 1979; and see Table I]. At this time, behavioral techniques were the only practical method available to estimate who reproduced with whom in most wild studies (but see early paternity analyses via protein electrophoresis in captive primates and DNA fingerprinting in free-ranging primates, e.g., Stern and Smith [1984] and Kuester et al. [1994], respectively; and early DNA fingerprinting studies in wild birds, e.g., Burke and Bruford [1987], Quinn et al. [1987], Wetton et al. [1987], Wrege and Emlen [1987]). However, mating behaviors are not easily observed in the wild, especially for arboreal primate species living in tropical forests, and behavioral observations are also problematic owing to the difficulties of observing covert mating behavior (e.g., extra-pair or extra-group copulations [Widdig et al., 2004] and ‘sneaky’ strategies [Berard et al., 1994; Kuester & Paul, 1992; Soltis et al., 2001]) and nocturnal behavior [Eberle & Kappeler, 2004; Schülke et al., 2004]. More particularly, in species where females mate with several males during the reproductive period (typically Old World species forming multimale–multifemale social groups), little is known concerning whether paternity is determined by male monopolization during critical time periods, female choice during these time periods, or sperm competition [Dixon, 1998, 2002; Engelhardt et al., 2006].

The improvement of genetic techniques has allowed researchers to evaluate how observed mating behaviors correlate with true paternities. It is clear from a number of studies that paternities cannot be determined from mating behavior alone. In wild populations, high-ranking males often produce more offspring than expected based on their mating success (e.g., long-tailed macaques *Macaca fascicularis*, De Ruiter et al. [1994]; patas monkeys *Erythrocebus patas*: Ohsawa et al. [1993]; savanna baboons *Papio cynocephalus*: Alberts et al. [2006]). In

contrast, in captive or semi-free ranging populations, high ranking males and/or successful mate guarders often produce fewer offspring than expected based on their mating success (e.g., mandrills: Setchell et al. [2005]; Barbary macaques: Paul et al. [1993]; Japanese macaques: Inoue et al. [1993]). This may reflect the fact that captive and semi-free ranging populations often live at unusually high densities in which surreptitious copulations may be more prevalent than in the wild [e.g., Berard et al., 1994]. At any rate, the difference between wild and captive populations demonstrates the flexibility of primate mating behavior, and highlights the fact that in species where females mate multiply, observers can never assume to see all copulations. Thus, even when mating behavior and paternity are well correlated [e.g., Altmann et al., 1996; Gust et al., 1998], pedigree construction requires much more detail than behavioral observations can provide, rendering the use of genetic tool necessary to establish kin relationships and inbreeding coefficients for each individual.

Genetic Analyses as a Tool in the Study of Inbreeding Depression: Applications and Limits

The earliest applications of molecular techniques to the study of primate behavior and genetic structure used allozyme markers [Stern & Smith, 1984]. As early as 1986, Smith used these techniques to determine paternities and compare life history-traits among inbred and non-inbred captive rhesus macaques (see Tables I and II). During the past two decades, microsatellite loci have become one of the favorite genetic markers for various applications, including parentage assignment and the study of inbreeding depression. In this way, paternity determination became more common in primatology in the 1990s and a variety of questions have been addressed [see for review Di Fiore, 2003]. The possible genetic techniques used for assigning paternity are widely explained and discussed elsewhere [Di Fiore, 2003; Martin et al., 1992], and we will not go further in the description of the methods used.

Problems Linked With Pedigree Data

One aim of paternity determination using genetic analysis is to reconstruct pedigrees. Indeed, the most straightforward approach for estimating inbreeding in natural populations is through the use of *well-resolved* pedigrees. However, such studies are relatively rare [Pemberton, 2004] because the required generations of deep and dense pedigrees represent a tremendous effort. Further, even relatively few gaps in a pedigree can dramatically reduce the researcher's ability to calculate accurate inbreeding coefficients [Marshall et al., 2002]. Indeed, for most primate populations, pedigree construction via molecular parentage analysis remains challenging and often yields incomplete pedigrees, in part because of the long generation time in primates. Furthermore, primate samples for genetic analyses (mainly feces and hairs for wild populations, blood or tissues from free-ranging or captive animals) have in most cases been collected only for one or two decades, resulting in pedigrees that are too shallow in time to draw accurate conclusions.

In primates, pedigree reconstructions are thus scarce. However, a complete five generation pedigree on a mandrill colony housed at CIRMF, Gabon, was reconstructed through paternity analyses using microsatellite loci [Charpentier et al., 2005c]. The resulting pedigree was complete as all potential sires were genotyped. Of 193 offsprings for whom paternity was determined, 58 (30%) were found to be inbred (range: $f = 0.0625-0.25$). However, under more naturalistic

conditions, inbreeding avoidance is likely to be more effective than in this mandrill colony, where males cannot migrate (although even in this colony, avoidance of consanguineous mating was detected between close relatives, i.e., for dyads with $R = 0.5$ [Charpentier et al., 2005c]). For example, in semi-free ranging rhesus macaques from Cayo-Santiago (Puerto Rico), genetically sampled since 1992, only 37 out of a total of 864 (4.2%) offspring with partial pedigrees available were inbred (i.e., born to related parents). Note that we considered only offspring with both parents genotyped and for whom the matriline was known. In these macaques, inbreeding levels among the 37 inbred offspring varied from $f = 0.004$ to 0.25 (A. Widdig, unpublished data), with the more inbred individuals resulting from parent-offspring reproductions. That is, in spite of the fact that the rhesus monkeys are not able to disperse away from the island population, they achieved a relatively low rate of inbreeding, which falls in the range reported by Ralls et al. [1986] for 28 wild species of birds and mammals.

In large populations experiencing natural male or female migration, inbred events are probably quite infrequent. However, the lack of deep pedigrees in most such populations does not allow any definitive conclusion about how accurately incomplete pedigrees reflect actual inbreeding levels [and see Marshall et al., 2002]. We thus suggest that results on inbreeding coefficients obtained from incomplete pedigrees should be carefully interpreted. Further analyses should also include information coming both from the pedigree and the genetical information directly, in the form of heterozygosity.

Heterozygosity-Fitness Correlations

An alternative approach to the use of pedigrees is to exploit the fact that inbreeding reduces heterozygosity [Hartl & Clark, 1997]. The consensus of a number of recent studies in several animal species is that associations between multilocus heterozygosity and the components of fitness are common [Britten, 1996]. In various animal species, heterozygosity has been shown to correlate positively with a number of fitness traits [Acevedo-Whitehouse et al., 2003; Amos et al., 2001; Coltman et al., 1998; Coulson et al., 1998, 1999; Gallardo et al., 2004; Hoffman et al., 2004; Mandal et al., 2004; Pujolar et al., 2005; Seddon et al., 2004; Slate et al., 2000]. These studies have generally invoked inbreeding depression as the likely underlying mechanism. However, several recent empirical and theoretical studies have questioned the validity of using multilocus heterozygosity as an estimate of inbreeding [Balloux et al., 2004; Coltman & Slate, 2003; Pemberton, 2004; Slate et al., 2004]. If neutral genetic diversity reflects genomewide heterozygosity at unlinked genes (i.e., inbreeding), the heterozygosity-fitness correlation should be equivalent across neutral markers, and not due to one or few loci linked with genes under selection (i.e., local effect). Balloux et al. [2004] therefore proposed that studies should look for heterozygosity-fitness associations that appear to be marker specific, by simply re-analyzing the heterozygosity-fitness correlation and fitting each locus individually.

To date, only one study has used microsatellite heterozygosity to test for inbreeding depression in a primate species [Charpentier et al., 2005a]. Here the authors tested whether the benefits of greater genetic diversity were due mainly to a genome-wide effect of inbreeding, or to heterosis at one or a few loci. Multilocus effects best explained the correlation between heterozygosity and reproductive success, indicating the occurrence of inbreeding depression in this mandrill colony. Furthermore, the authors were able to compare genetical information obtained both from a pedigree [see Charpentier et al., 2006] and microsatellite heterozygosity. They found that pedigree information was limited

by its depth; that is, it can only provide information about the period of time covered by the pedigree. Specifically, in this case, the pedigree did not reflect the fact that the 15 founder mandrills came from two distinct areas in Gabon, and that outbred mating consequently occurred in the first generations [Charpentier et al., 2005a].

The Future

We have shown that accurate pedigree data are difficult to obtain, but should be pursued whenever possible. At least, a complete two-generation pedigree should provide estimates of f good enough to detect inbreeding and study its effects. The use of microsatellite data to estimate inbreeding appears one of the best current alternatives to pedigree data, if precautions are taken as follow. First, neutral diversity should reflect genomewide heterozygosity at unlinked genes, if the heterozygosity-fitness correlation is equivalent across neutral markers. Second, for inbreeding to be detectable with the use of neutral heterozygosity, variation in inbreeding in the studied population is necessary [Balloux et al., 2004]. However, an ideal primate data set would include an estimate of genetic diversity through both a pedigree and a microsatellite data set. This would allow researchers to estimate both the real kinship links between individuals that reproduce in a given population, as well as identify potential outbreeding events in cases where reproductive individuals come from genetically differentiated populations. Third, in species where the whole genome has been sequenced (rhesus macaques and chimpanzees *Pan troglodytes*), the genomic data will open new opportunities to genotype and analyze a large number of both selected and neutral markers to estimate precisely inbreeding.

CONCLUSIONS

The implications of inbreeding depression analysis for conservation are very important, because modern genetic tools provide powerful methods to demonstrate the consequences of habitat destruction on the genetic structure of primate populations [Andayani et al., 2001; Evans et al., 2001; Fredsted et al., 2004; Goossens et al., 2006]. A decrease in genetic variability is likely to reduce the capacity for adaptation to changing and unpredictable environments, capacity which is proportional to the genetic variance [Fisher, 1930]. In contrast, high genetic variability theoretically maximizes a population's ability to remain viable in the face of unpredictable environmental challenges. We therefore strongly encourage primatologists, particularly those working on endangered species, to obtain molecular data and to estimate both the extent of inbreeding and its variance in their study populations. Studies of semi-free ranging and captive populations will often be as valuable as those of wild populations, because they can provide insight into the extent to which animals will avoid inbreeding under various conditions, and into specific manifestations of inbreeding depression in different species. Inbreeding analyses should involve either pedigree construction or analyses of heterozygosity at microsatellite loci, or both. We also urge researchers to evaluate the severity of inbreeding depression through an analysis of various fitness estimates or correlates.

ACKNOWLEDGMENTS

Authors are grateful to Drs. Joanna Setchell and Franck Prugnolle for their helpful comments and discussions on an earlier version of the manuscript.

Support was provided by a Marie-Curie Outgoing Fellowships to MJEC, an Emmy Noether grant by the German Science Foundation (DFG) to AW (WI 1801/1-1 and 2-1) and the National Science Foundation (IOB-0322613, BCS-0323553) to SCA. Work on Cayo-Santiago was supported by Grant Number CM-5 P40 RR003640-20 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH. We finally thank the Caribbean Primate Research Center and Dr. Edmundo Kraiselburd for granting us access to Cayo-Santiago, and Dr. Melissa Gerald and Edgar Davila for access to demographic data.

REFERENCES

- Acevedo-Whitehouse K, Gulland F, Grieg D, Amos W. 2003. Inbreeding: disease susceptibility in California sea lions. *Nature* 422:35.
- Alberts SC, Altmann J. 1995. Balancing costs and opportunities: dispersal in male baboons. *Am Nat* 145:279–306.
- Alberts SC, Buchan JC, Altmann J. 2006. Sexual selection in wild baboons: from mating opportunities to paternity success. *Anim Behav* 72:1177–1196.
- Altmann J, Alberts SC. 2003. Intraspecific variability in fertility and offspring survival in a nonhuman primate: behavioral control of ecological and social sources. In: Wachter KW, Bulatao RA, editors. *Offspring: the biodemography of fertility and family behavior*. Washington, DC: National Academy Press. p 140–169.
- Altmann J, Alberts SC, Haines, SA, Dubach J, Muruthi P, Coote T, Geffen E, Cheesman DJ, Mututua RS, Saiyalel SN, Wayne RK, Lacy RC, Bruford M. 1996. Behavior predicts genetic structure in a wild primate group. *Proc Natl Acad Sci USA* 93: 5797–5801.
- 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 Roy Soc Lond B: Biol Sci* 268:2021–2027.
- Andayani N, Morales JC, Forstner MRJ, Supriatna J, Melnick DJ. 2001. Genetic variability in mtDNA of the silvery gibbon: implications for the conservation of a critically endangered species. *Conserv Biol* 15: 770–775.
- Balloux F, Amos W, Coulson T. 2004. Does heterozygosity estimate inbreeding in real populations? *Mol Ecol* 13:3021–3031.
- Barret SCH, Charlesworth D. 1991. Effect of a change in the level of inbreeding on the genetic load. *Nature* 352:522–524.
- Beehner JC, Nguyen N, Wango EO, Alberts SC, Altmann J. 2006a. The ecology of conception and pregnancy failure in wild baboons. *Horm Behav* 49:688–699.
- Beehner JC, Onderdonk DA, Alberts SC, Altmann J. 2006b. The ecology of conception and pregnancy failure in wild baboons. *Behav Ecol* 17:741–750.
- Berard JD, Nürnberg P, Epplen JT, Schmidtke J. 1994. Alternative reproductive tactics and reproductive success in male rhesus macaques. *Behaviour* 129:177–201.
- Blouin SF, Blouin M. 1988. Inbreeding avoidance behaviors. *Trends Ecol Evol* 3:230–233.
- Britten HB. 1996. Meta-analyses of the association between multilocus heterozygosity and fitness. *Evolution* 50:2158–2164.
- Bulger J, Hamilton WJ. 1988. Inbreeding and reproductive success in a natural Chacma baboon (*Papio cynocephalus ursinus*) population. *Anim Behav* 36:574–578.
- Burke T, Bruford MW. 1987. DNA fingerprinting in birds. *Nature* 327:149–152.
- Bush M, Montali RJ, Kleiman DG, Randolph J, Abramowitz MD, Evans RF. 1980. Diagnosis and repair of familial diaphragmatic defects in golden lion tamarins. *J Am Vet Med Assoc* 177:858–862.
- Chalifoux LV, Elliott MW. 1986. Congenital anomalies in two neonatal tamarins (*Saguinus oedipus* and *Saguinus fuscicollis*). *J Med Primatol* 15:329–337.
- Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Sys* 18: 237–268.
- Charlesworth D, Charlesworth B. 1999. The genetic basis of inbreeding depression. *Genet Res* 74:329–340.
- Charpentier M, Setchell JM, Prugnolle F, Knapp LA, Wickings EJ, Peignot P, Hossaert-McKey M. 2005a. Genetic diversity and reproductive success in mandrills (*Mandrillus sphinx*). *Proc Natl Acad Sci USA* 102:16723–16728.
- Charpentier M, Hossaert-McKey M, Wickings EJ, Peignot P. 2005b. Consequences of a one-male harem reproductive system and inbreeding in a captive group of *Cercopithecus solatus*. *Int J Primatol* 26:697–710.

- Charpentier M, Peignot P, Hossaert-McKey M, Gimenez O, Setchell JM, Wickings EJ. 2005c. Constraints on control: factors influencing reproductive success in male mandrills (*Mandrillus sphinx*). *Behav Ecol* 16: 614–623.
- Charpentier M, Setchell JM, Prugnolle F, Wickings EJ, Peignot P, Balloux F, Hossaert-McKey M. 2006. Life history correlates of inbreeding depression in mandrills (*Mandrillus sphinx*). *Mol Ecol* 15:21–28.
- Coltman DW, Slate J. 2003. Microsatellite measures of inbreeding: a meta-analysis. *Evolution* 57:971–983.
- Coltman DW, Bowen WD, Wright JM. 1998. Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proc Roy Soc Lond B: Biol Sci* 265:803–809.
- Constable JL, Ashley MV, Goodall J, Pusey AE. 2001. Noninvasive paternity assignment in Gombe chimpanzees. *Mol ecol* 10:1279–1300.
- Coulson TN, Pemberton JM, Albon SD, Beaumont M, Marshall TC, Slate J, Guinness FE, Clutton-Brock TH. 1998. Microsatellites reveal heterosis in red deer. *Proc Roy Soc Lond B: Biol Sci* 267: 489–495.
- Coulson TN, Albon SD, Slate J, Pemberton JM. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution* 53: 1951–1960.
- Crawford MH, O'Rourke DH. 1978. Inbreeding, lymphoma, genetics and morphology of the *Papio hamadryas* colony of Sukhumi. *J Med Primatol* 7:355–360.
- Crawford MH, O'Rourke DH, Dykes DD, Yakovleva LA, Voevodin AF, Lapin B, Polesky HF. 1984. Inbreeding, heterozygosity, and lymphoma risk among the baboons (*Papio hamadryas*) of Sukhumi, USSR. *Am J Primatol* 6:143–153.
- Crawford MH, Devor EJ, O'Rourke DH, Yakovleva LA, Voevodin AF, Lapin B. 1987. Genetics of primate lymphomas in a baboon (*Papio hamadryas*) colony of Sukhumi, USSR. *Genetica* 73:145–157.
- Crnokrak P, Roff DA. 1999. Inbreeding depression in the wild. *Heredity* 83:260–270.
- De Ruiter JR, van Hooft JARAM, Scheffrhan W. 1994. Social and genetic aspects of paternity in wild long-tailed macaques (*Macaca fascicularis*). *Behaviour* 129: 203–223.
- Di Fiore A. 2003. Molecular genetic approaches to the study of primate behavior, social organization, and reproduction. *Yearbook Phys Anthropol* 46:62–99.
- Dietz JM, Baker AJ. 1993. Polygyny and female reproductive success in golden lion tamarins, *Leontopithecus rosalia*. *Anim Behav* 46:1067–1078.
- Dixson A. 2002. Sexual selection by cryptic female choice and the evolution of primate sexuality. *Evol Anthropol* 11(Suppl. 1): 195–199.
- Dixson AF. 1998. Primate sexuality: comparative studies of the prosimians, monkeys, apes, and human beings. Oxford: Oxford University Press. 560p.
- Eberle M, Kappeler PM. 2004. Sex in the dark: determinants and consequences of mixed male mating tactics in *Microcebus murinus*, a small solitary nocturnal primate. *Behav Ecol Sociobiol* 57:77–90.
- Engelhardt A, Heistermann M, Hodges JK, Nuernberg P, Niemitz C. 2006. Determinants of male reproductive success in wild long-tailed macaques (*Macaca fascicularis*)-male monopolization, female mate choice or post-copulatory mechanism? *Behav Ecol Sociobiol* 59:740–752.
- Evans BJ, Supriatna J, Melnick DJ. 2001. Hybridization and population genetics of two macaque species in Sulawesi, Indonesia. *Evolution* 55:1686–1702.
- Fisher RA. 1930. The genetical theory of natural selection. Oxford: Clarendon Press. 318p.
- Frankham R. 1995. Inbreeding and extinction: a threshold effect. *Conserv Biol* 9:792–799.
- Frankham R. 2003. Genetics and conservation biology. *CR Biol* 326:S22–S29.
- Frankham R, Ballou JD, Briscoe DA. 2002. Introduction to conservation genetics. Cambridge: Cambridge University Press. 617p.
- Fredsted T, Pertoldi C, Olesen JM, Eberle M, Kappeler PM. 2004. Microgeographic heterogeneity in spatial distribution and mtDNA variability of gray mouse lemurs (*Microcebus murinus*, Primates: Cheirogaleidae). *Behav Ecol Sociobiol* 56:393–403.
- Gallardo JA, Garcia X, Lhorente JP, Neira R. 2004. Inbreeding and inbreeding depression of female reproductive traits in two populations of Coho salmon selected using BLUP predictors of breeding values. *Aquaculture* 234:111–122.
- Goossens B, Chikhi L, Ancrenaz M, Lackman-Ancrenaz I, Andau P, Bruford MW. 2006. Genetic signature of anthropogenic population collapse in orang-utans. *Plos Biol* 4: 285–291.
- Gust DA, McCaster T, Gordon TP, Gergits WF, Casna NJ, Mc Clure HM. 1998. Paternity in sooty mangabeys. *Int J Primatol* 19: 83–94.
- Hartl DL, Clark AG. 1997. Principles of population genetics. Sunderland: Sinauer Associates. 542p.
- 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.
- Inoue M, Mitsunaga F, Nozaki M, Ohsawa H, Takenaka A, Sugiyama Y, Shimizu K, Takenaka O. 1993. Male-dominance rank and reproductive success in an enclosed group of Japanese macaques—with special reference to post-conception mating. *Primates* 34:503–511.
- Isachenko EF, Nayudu PL, Isachenko VV, Nawroth F, Michelmann HW. 2002. Congenitally caused fused labia in the common marmoset (*Callithrix jacchus*). *J Med Primatol* 31:350–355.
- Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. *Trends Ecol Evol* 17:230–241.
- Kuester J, Paul A. 1992. Influence of male competition and female mate choice on male mating success in Barbary macaques (*Macaca sylvanus*). *Behaviour* 120:192–217.
- Kuester J, Paul A, Arnemann J. 1994. Kinship, familiarity and mating avoidance in Barbary macaques, *Macaca sylvanus*. *Anim Behav* 48:1183–1194.
- Lacy RC, Petric A, Warneke M. 1993. Inbreeding and outbreeding in captive populations of wild animal species. In: Thornhill NW, editor. *The natural history of inbreeding and outbreeding*. Chicago: University of Chicago Press. p 352–374.
- Mandal A, Pant KP, Rout PK, Roy R. 2004. Effects of inbreeding on lamb survival in a flock of Muzaffarnagari sheep. *Asian Austr J Anim* 17:594–597.
- Marshall TC, Coltman DW, Pemberton JM, Slate J, Spalton JA, Guinness FE, Smith JA, Pilkington JG, Clutton-Brock TH. 2002. Estimating the prevalence of inbreeding from incomplete pedigrees. *Proc Roy Soc Lond B: Biol Sci* 269:1533–1539.
- Martin RD, Dixson AF, Wickings EJ. 1992. Paternity in primates: genetic tests and theories. Implications of human DNA fingerprinting. Basel: Karger. 288p.
- Nakamichi M, Nobuhara H, Nobuhara T, Nakahashi M, Nigi H. 1997. Birth rate and mortality rate of infants with congenital malformations of the limbs in the Awajishima free-ranging group of Japanese monkeys (*Macaca fuscata*). *Am J Primatol* 42:225–234.
- Newman D, Pilson D. 1997. Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution* 51:354–362.
- Noble SJ, Chesser RK, Ryder OA. 1990. Inbreeding effects in captive populations of Ruffed Lemurs. *Hum Evol* 5:283–291.
- Ohsawa H, Inoue M, Takenaka O. 1993. Mating strategy and reproductive success of male pata monkeys (*Erythrocebus patas*). *Primates* 34:533–544.
- Packer C. 1979. Inter-troop transfer and inbreeding avoidance in *Papio anubis*. *Anim Behav* 27:1–36.
- Paul A, Kuester J, Timme A, Arnemann J. 1993. The association between rank, mating effort, and reproductive success in male Barbary macaques (*Macaca sylvanus*). *Primates* 34:491–502.
- Peer K, Taborskyi M. 2005. Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. *Evolution* 59:317–323.
- Pemberton J. 2004. Measuring inbreeding depression in the wild: the old ways are the best. *Trends Ecol Evol* 19:613–615.
- Perrin N, Mazalov V. 1999. Dispersal and inbreeding avoidance. *Am Nat* 154:282–292.
- Perrin N, Mazalov V. 2000. Local competition, inbreeding, and the evolution of sex-biased dispersal. *Am Nat* 155:116–127.
- Prugnolle F, Choisy M, Théron A, Durand P, De Meeus T. 2004. Sex-specific correlation between heterozygosity and clone size in a trematode *Schistosoma mansoni*. *Mol Ecol* 13:2859–2864.
- Pujolar JM, Maes GE, Vancoillie C, Volckaert FAM. 2005. Growth rate correlates to individual heterozygosity in the European eel *Anguilla anguilla* L. *Evolution* 59:189–199.
- Pusey A. 1990. Mechanisms of inbreeding avoidance in nonhuman primates. In: Feerman JR, editor. *Pedophilia*. New York: Springer. p 201–220.
- Pusey AE, Packer C. 1987. Dispersal and philopatry. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Struhsaker TT, editors. *Primate societies*. Chicago: The University of Chicago Press. p 250–266.
- Pusey A, Wolf M. 1996. Inbreeding avoidance in animals. *Trends Ecol Evol* 11:201–206.
- Quinn TW, Quinn JS, Cooke F, White BN. 1987. DNA marker analysis detects multiple paternity and maternity in single broods of the lesser snow goose. *Nature* 326:392–394.
- Ralls K, Ballou J. 1982. Effects of inbreeding on infant mortality in captive primates. *Int J Primatol* 3:491–505.
- Ralls K, Harvey PH, Lyles AM. 1986. Inbreeding in natural populations of birds and mammals. In: Soulé ME, editor. *Conservation biology—the science of scarcity and diversity*. Sunderland, MA: Sinauer Associates. p 35–56.
- Rawlins RG, Kessler MJ. 1983. Congenital and hereditary anomalies in the rhesus monkeys (*Macaca mulatta*) of Cayo Santiago. *Teratology* 28:169–174.
- Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392:491–494.

- Schülke O, Kappeler PM, Zischler H. 2004. Small tested size despite high extra-pair paternity in the pair-living nocturnal primate *Phaner furcifer*. *Behav Ecol Sociobiol* 55:293–301.
- Seddon N, Amos W, Mulder RA, Tobias JA. 2004. Male heterozygosity predicts territory size song structure and reproductive success in a cooperatively breeding bird. *Proc Roy Soc Lond B: Biol Sci* 271: 1823–1829.
- Setchell JM, Charpentier M, Wickings EJ. 2005. Sexual selection, age specific reproductive output and reproductive careers in mandrills (*Mandrillus sphinx*). *Behav Ecol Sociobiol* 58:474–485.
- Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proc Roy Soc Lond B: Biol Sci* 267: 1657–1662.
- Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE, McEwan JC. 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* 93:255–265.
- Smith DG. 1986. Incidence and consequences of inbreeding in three captive groups of rhesus macaques (*Macaca mulatta*). In: Benirschke I, editor. *Primates. The road to self-sustaining populations*. New York: Springer-Verlag. p 857–874.
- Smith DG. 1995. Absence of deleterious effects of inbreeding in rhesus macaques. *Int J Primatol* 16:855–870.
- Soltis J, Thomsen R, Takenaka O. 2001. The interaction of male and female reproductive strategies and paternity in wild Japanese macaques, *Macaca fuscata*. *Anim Behav* 62: 485–494.
- Stern BR, Smith DG. 1984. Sexual behaviour and paternity in three captive groups of rhesus monkeys (*Macaca mulatta*). *Anim Behav* 32:23–32.
- Stills HF, Bullock BC. 1981. Congenital defects of squirrel monkeys (*Saimiri sciureus*). *Vet Pathol* 18:29–36.
- Thünken T, Bakker TCM, Baldauf SA, Kullmann H. 2007. Active inbreeding in a cichlid fish and its adaptive significance. *Curr Biol* 17:225–229.
- van Oosterhout C, Zijlstra WG, van Heuven MK, Brakefield PM. 2000. Inbreeding depression and genetic load in laboratory metapopulations of the butterfly *Bicyclus anynana*. *Evolution* 54:218–225.
- Waller DM. 1993. The statics and dynamics of mating system evolution. In: Thornhill N, editor. *The natural history of inbreeding and outbreeding*. Chicago: University of Chicago Press. p 97–117.
- Wang J. 2000. Effects of population structures and selection strategies on the purging of inbreeding depression due to deleterious mutations. *Genet Res Camb* 76: 75–86.
- Wetton JH, Carter RE, Parkin DT, Walters D. 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* 327:147–149.
- Widdig A, Bercovitch FB, Streich WJ, Sauer- mann U, Nürnberg P, Krawczak M. 2004. A longitudinal analysis of reproductive skew in male rhesus macaques. *Proc Roy Soc Lond B: Biol Sci* 271:819–826.
- Willis JH. 2000. The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* 53:1678–1691.
- Wrege PH, Emlen ST. 1987. Biochemical determination of paternal uncertainty in white-fronted bee-eaters. *Behav Ecol Sociobiol* 20:153–160.