

Maternal condition determines infant sex in black howler monkeys (*Alouatta pigra*) in Mexico

Pedro Américo D. Dias^{1*}, Alejandro Coyohua-Fuentes¹, Domingo Canales-Espinosa¹ and Ariadna Rangel-Negrín¹

¹Laboratorio de Ecología del Comportamiento de Primates, Instituto de Neuroetología, Universidad Veracruzana, Av. Dr. Luis Castelazo Ayala S/N, Colonia Industrial Animas, CP 91190, Xalapa, Veracruz, Mexico.

*paddias@hotmail.com

Abstract

There is growing evidence that skewed sex ratios at birth may be influenced by maternal condition around the moment of conception. During 7 years, we monitored 11 groups of black howler monkeys (*Alouatta pigra*) comprising 45 adult females, and analyzed whether off-spring sex was related to maternal condition around conception measured as: 1) the amount of ingested fruit; 2) the occurrence of lactation; 3) the sex of the previous infant; 4) the survival of the previous infant; 5) habitat quality. The probability to produce a son was higher for females that were not lactating around conception. However, this effect was probably determined by a positive relationship between male infant mortality and the conception of sons. Our results call for research on the physiological mechanisms underlying these patterns to better understand the adaptive significance of sex allocation in black howler monkeys.

Resumen

La condición materna determina el sexo de los infantes en monos aulladores negros (*Alouatta pigra*) en México

La evidencia de que el sesgo en la proporción sexual de las crías parece relacionarse con la variación en la condición física de las hembras cerca del momento de la concepción es cada vez más contundente. Durante 7 años, monitoreamos 11 grupos de monos aulladores negros (*Alouatta pigra*) y un total de 45 hembras adultas, para determinar si el sexo de sus crías estaba relacionado con su condición al momento de la concepción medida como: 1) la cantidad de fruta ingerida; 2) la ocurrencia de lactancia; 3) el sexo de la cría anterior; 4) la supervivencia de la cría anterior; 5) la calidad del hábitat. La probabilidad de producir un hijo fue mayor en hembras que no estaban lactando al momento de la concepción. Sin embargo, este efecto fue probablemente determinado por una relación positiva entre la mortalidad de las crías macho y la concepción de machos. Necesitamos investigar los mecanismos fisiológicos subyacentes a estos resultados para comprender el valor adaptativo de la variación en la determinación del sexo en los monos aulladores negros.

Key words

Palabras clave

Habitat quality, lactation, sex allocation, sex ratio.

Calidad del hábitat, determinación del sexo, lactancia, proporción sexual.

INTRODUCTION

Why do some animal species have skewed sex ratios at birth? This question has been the focus of abundant research, and sex allocation theory continues to be an important topic in behavioral ecology (Cameron 2004; Silk & Brown 2004). Whereas the ultimate evolutionary significance of sex ratios that deviate from unity is still disputed, there is consensus over the importance of understanding variation in sex ratios, as sex ratios influence many dimensions of animal reproductive strategies, behavior and demography (Clutton-Brock & Iason 1986; West & Sheldon 2002; Silk & Brown 2004).

Attention has recently been drawn to the study of proximate explanations for sex ratios at birth, resulting in growing evidence that the physiological mechanism for sex determination is linked to the maternal condition around the time of conception: variation in intra-uterine glucose levels (Cameron 2004), fluctuation in hormone levels (James 2008), and asynchrony in embryo development (Krackow 1995), which are related to maternal condition, explain variation in infant sex at birth in several species. For instance, in tammar wallabies (*Macropus eugenii*), high access to food resources causes increased glucose levels in females, which in turn favors the production of daughters (Schwanz & Robert 2014). In several bird species, elevated levels of circulating glucocorticoids also skew sex ratios at birth in favor of females (e.g., Japanese quails, *Coturnix japonica*: Love *et al.* 2005; white-crowned sparrows, *Zonotrichia leucophrys*: Bonier *et al.* 2007). Despite such progress, most studies about sex determination in wild mammals still rely on indirect measures of maternal condition around the time of conception, as it remains difficult to monitor female reproductive physiology non-invasively, and long-term datasets are required to perform empirical tests of proximate or ultimate hypothesis of sex bias in long-lived animals. Examples of indirect measures of maternal condition include rainfall (red deer, *Cervus elaphus*: Kruuk *et al.* 1999; black rhinoceros: *Diceros bicornis minor*: Law *et al.* 2014), dominance rank (primates: van Schaik & Hrdy 1991; Schino 2004), age (olive baboons, *Papio anubis*: Packer *et al.* 2000; ungulates: Saltz 2001; bighorn sheep, *Ovis canadensis*: Martin & Festa-Bianchet 2011), lactation around the time of conception (American bison, *Bison*

bison: Rutberg 1986), and sex of preceding offspring (bighorn sheep: Hogg *et al.* 1992).

Biased sex ratios at birth have been observed in several monotocous primate species. Most studies of sex allocation in primates have used female dominance rank as a proxy to maternal condition, based on evidence that dominant females have priority of access to resources (Silk & Brown 2004), which should grant them better body condition compared to more subordinate females. At the species level, variation in female dominance rank has been found to explain differences in the number of male and female progeny in some studies, but overall there is no consistent relationship between dominance rank and offspring sex (Schino 2004; Silk *et al.* 2005). This inconsistency may be due to the use of an inaccurate measure of female condition (e.g., dominance rank does not always correlate positively with body condition: Dittus 1998) or to reduced statistical power in most studies (Schino 2004). Only three studies have used direct measures of female condition around conception. In toque macaques (*Macaca sinica*) mothers with higher body weight have more sons, whereas those in moderate condition give birth to more daughters, but both effects are expressed most strongly among mothers of high rank (Dittus 1998). In contrast, infant sex in olive baboons is not related to maternal condition or maternal dominance rank (Silk & Strum 2007); and fatter rhesus macaque (*Macaca mulatta*) females do not give birth to infants of a particular sex (Berman 1988). These three species have polygynous mating systems, female philopatry, and matrilineal social relationships, which are biological traits that have been used to explain variation in sex allocation in mammals (Trivers & Willard 1973; Clark 1978; Silk 1983). Thus, as predicted by evolutionary theory (Trivers & Willard 1973), covariance between sex ratios at birth and maternal condition would be expected to occur. Therefore, there is also inconsistency among the results of studies that have used direct measures of maternal condition, highlighting the importance of further exploration of interindividual variation in birth sex ratios in primates.

We have previously reported a 2:1 male to female sex ratio at birth in a population of black howler monkeys (*Alouatta pigra*: Dias *et al.* 2015), a proportion that differs significantly from unity (Binomial probability test, $P = 0.002$, $N = 96$ births). The aim of the present

study was to determine which factors could explain this skew. In this population, black howler monkeys live in groups of two to 13 individuals, including one to four adult males and one to four adult females, with an adult sex ratio that varies from 0.5 to 4 females per male (Dias *et al.* 2015). In this species no clear dominance hierarchies can be discerned among females and among males, although usually one male monopolizes reproduction (Van Belle *et al.* 2008, 2011). Both sexes leave their natal groups, and secondary dispersal has been also observed (Van Belle *et al.* 2009a; Dias *et al.* 2015). Mean lactation length is 14 months (Dias *et al.* 2011) and interbirth interval is 16 months (Dias *et al.* 2015, 2016). As gestation length is six months (Van Belle *et al.* 2009b) females may conceive while still lactating her about 10-month-old infant. Births are clustered in the wet season (71.9% of all births), indicating that conceptions are more frequent in the dry season, when the availability of energy-rich foods is lower (Schaffner *et al.* 2012).

The aim of the present study was to determine if offspring sex depended on maternal condition around conception in black howler monkeys. Maternal condition should be a function of both the investment in dependent offspring and the availability of resources for reproduction around conception. We assumed that the production and rearing of infant males is costlier than

that of infant females because in black howler monkeys the body mass of adult males is 22-41% greater than that of females (Kelaita *et al.* 2011), and adult body size is reached at 5 yr by males and 4 yr by females (Kitchen 2000). Furthermore, in other howler monkey species, male weight gain during the first year of life is faster than in females (Froehlich *et al.* 1981; Leigh 1994). Therefore, as documented for other mammals (Gomenadio *et al.* 1990), energetic investment in rearing male infants should be higher. We therefore expected that females in better condition would produce males, and the probability of conceiving a male would be higher when females had no dependent infants (i.e., were not lactating), when the previous infant was a female (i.e., lower energetic investment in rearing), and when the consumption of high-quality food resources was higher (fruits: Behie & Pavelka 2015). Howler monkeys currently live under very contrasting ecological conditions as a consequence of anthropogenic habitat disturbance (Arroyo-Rodríguez & Dias 2010). Habitat attributes could affect female behavior and physiology through a number of mechanisms, including variation in disease risk or stress levels (Rangel-Negrín *et al.* 2014; Canales-Espinosa *et al.* 2015). Therefore, we also explored the possibility that habitat quality indirectly affected sex determination in black howler monkeys.

METHODS

Ethical note

Our research protocols complied with the Mexican legal requirements and were approved by permits approved by SEMARNAT/SGPA/DGVS/01273/06 and SEMARNAT/SGPA/DGVS/04949/07.

Study area and subjects

The study was conducted in the State of Campeche, Mexico. From February 2006 to February 2012 we studied 11 groups of black howler monkeys in 11 different forest fragments (Dias *et al.* 2014, 2015; Figure 1). Each year, each group was sampled at least twice in each climatic season (i.e. dry and rainy season). One group could only be sampled from 2006 to 2010, because the forest fragment in which it lived was logged. Demographic sampling

during each season encompassed a mean (\pm SD) of 67 (\pm 5.4) days (range = 57-73 days). We accumulated a total of 10,083 sampling days, with a mean (\pm SD) of 918.5 (\pm 125.3) sampling days per group. All monkeys were individually recognizable by researchers via ankle bracelets or their natural anatomical and physiognomic characteristics, such as body size and proportions, scars, broken fingers, and genital morphology and pigmentation.

Each time a newborn was observed, its sex was recorded. The contrast of the white scrotum of male infants against their black pelage allows for the easy discrimination of sex from the moment of birth. Because infants (i.e. individuals < 12 months) are highly dependent on their mothers and cannot survive alone, when an infant could not be located for more than two sampling days, but the mother was still in the group, we considered that it died.

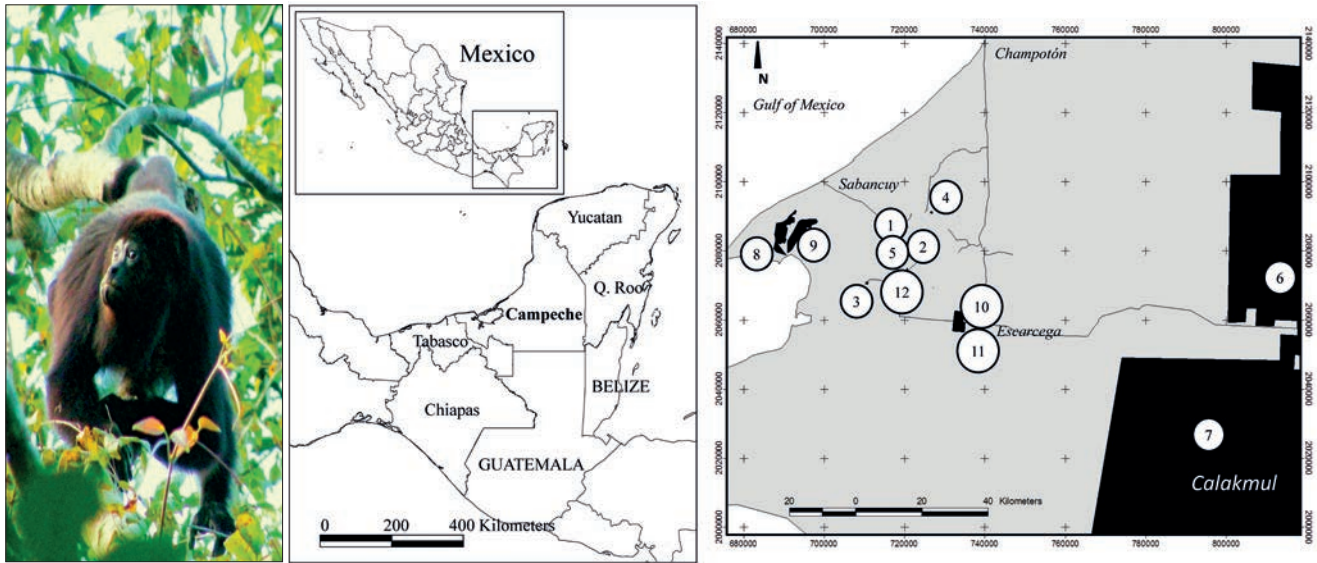


Figure 1. Location of the 11 groups of black howler monkeys studied in the state of Campeche, Mexico. Groups: 1 = AA Álamo; 2 = Chicbul; 3 = Manantiales; 4 = Oxcabal; 5 = R Álamo; 6 = Calakmul N; 7 = Calakmul S; 8 = Calaxchil; 9 = T61 Calax; 10 = Tormento N; 11 = Tormento S.

Dietary data

Dietary data was collected for each adult female ($N = 45$) in each group as described by Dias *et al.* (2011, 2014). Briefly, each female was observed during 1-hr focal samples, in which the duration of feeding episodes was recorded. Time spent feeding from fruits was multiplied by feeding rates to obtain an estimation of the amount of ingested fruit (in grams) as described in Dias *et al.* (2014). From these data, we calculated percentages of ingested fruit weight per female per sampling month. Mean (\pm SD) monthly sampling time per female was 3.2 ± 5.8 h.

Characterization of habitat quality

It has been previously proposed that habitat quality for howler monkeys is a function of the availability of habitat (both at a forest fragment and at a landscape scale), large trees, and plant species; and of population density (e.g., Arroyo-Rodríguez & Dias 2010; Arroyo-Rodríguez *et al.* 2013; Dias *et al.* 2014, 2015). Based on the methods described by Dias *et al.* (2013, 2014, 2015) we calculated these attributes for the fragment in which each sampled group lived (Table 1). With the resulting data we performed a cluster analysis to identify similarities among groups in the quality of their habitats. Two main clusters of quality of the habitat where the sampled

groups lived were identified (Figure 2), which differed significantly in terms of fragment size (one-way ANOVA $F_{1,9} = 16.7$, $P = 0.002$), amount of habitat in surrounding landscape ($F_{1,9} = 54.4$, $P < 0.001$), basal area of trees ($F_{1,9} = 22.3$, $P = 0.001$), plant species richness ($F_{1,9} = 7.1$, $P = 0.026$), and population density ($F_{1,9} = 6.7$, $P = 0.029$). Therefore, in high-quality fragments the availability of habitat, large trees and plant species was higher than in low-quality fragments, whereas population density was lower.

Statistical analysis

Given that gestation in black howler monkeys lasts *ca.* 184 days (Van Belle *et al.* 2009b), for each recorded birth we quantified the percentage of ingested fruit in the diet of each female and the occurrence of lactation (assessed through the observation of nipple contact: Rijt-Plooi & Plooi 1987) on the seventh month before birth date to characterize maternal state around conception. We additionally recorded the sex of the previous infant and whether it survived, and the quality of the fragment in which mothers lived. We performed generalized linear mixed models (GLMM) to explore relationships between predictive variables (Rabe-Hesketh *et al.* 2005). For categorical variables, such as the occurrence of lactation around conception or sex of previ-

Table 1. Attributes of the fragments occupied by 11 black howler monkey groups in Campeche, Mexico, studied between 2006 and 2012

Group	Fragment size (ha) ^a	Amount of habitat in surrounding landscape (ha) ^b	Sum of basal areas of trees (cm) ^c	Plant species richness ^c	Population density (ind/km ²) ^d
Low-quality fragments					
AA Álamo	35.3	193.1	1,334.8	17	52.1
Chicbul ^e	5	33.7	1,436.2	16	120.0
Manantiales	50	268.5	1,626.1	22	28.0
Oxcabal	7	52.6	1,436.2	5	62.6
R Álamo	96	161.2	1,656.2	25	3.5
Mean (±SE)	38.7±16.7	141.8±44	1,497.9±61.5	17.0±3.4	53.2±19.6
High-quality fragments					
Calakmul N	51,503	1,190.8	3,003.9	22	5.3
Calakmul S	140,000	1,200	3,353.7	33	6.3
Calaxchil	3,000	985.6	2,577.9	24	1.9
T61Calax	300	540.1	2,237.9	25	2.5
Tormento N	600	1,017.4	1,920.8	26	10.1
Tormento S	800	1,097.5	2,650.4	29	15.9
Mean (±SE)	32,700.5±22,983.1	1,005.2±99.6	2,624.1±210	26.5±1.6	7±2.2

^a This variable was log-transformed before analysis.
^b Measured in 1,200 ha landscapes (Rangel-Negrín *et al.* 2014).
^c Determined through ten 50 x 2 m linear transects inside each group's home range (Gentry 1982).
^d Calculated through direct counts of individuals in each fragment (Dias *et al.* 2013).
^e This group was only sampled from 2006 to 2010.

ous infant, we used GLMM with a binomial error structure and logit-link function. The percentage of ingested fruit in the diet of each female around conception was arcsine-square-root transformed and analyzed with Gaussian error structure and identity link function. We diagnosed model validity through distribution of residuals and quantile-quantile plots to verify normality and residuals plotted against predicted values to assess homogeneity.

For modeling predictors of infant sex we used GLMM with a binomial error structure and logit-link function, with infant sex as a binomial response variable (i.e., male/female) and the following predictive variables: 1) percentage of ingested fruit in the diet of each female around conception; 2) occurrence of lactation around conception; 3) sex of

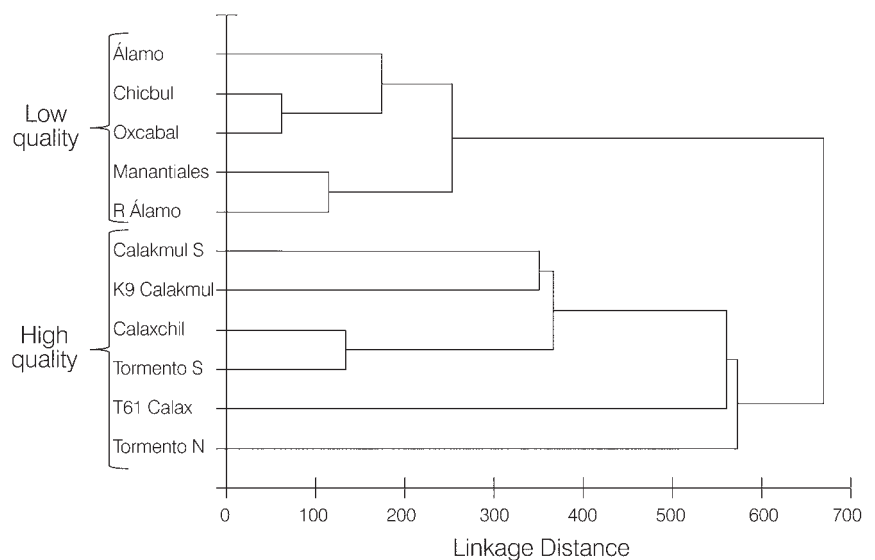


Figure 2. Similarity among habitats in attributes that define habitat quality for black howler monkeys in Campeche, Mexico, studied between 2006 and 2012.

the previous infant; 4) survival of previous infant; 5) habitat quality. To assess collinearity among predictive variables we used the 'perturb' package (Hendrickx 2015) in R (R Development Core Team 2016). We

found that sex and survival of the previous infant had significant effects on parameter estimations of other predictors, so they were excluded from the final model. Female identity was included as a random factor in all

GLMMs to account for repeated observations of females contributing more than one birth to the data set. GLMMs were performed with SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

We recorded a total of 76 births for which mothers were observed in the putative conception month. Females had a frugivorous diet at conception (mean \pm SD = 61.2 \pm 13.4% of fruit in overall diet), with 58% of conceptions being associated with diets including 50-70% of fruit. Females were usually not lactating (72.4% of births) around conception. For 77.1% of births that were analyzed, the previous infant was a male and 48.7% of previous infants were dead by conception time. The sex of the previous infant significantly predicted its survival ($F_{1,74} = 13.3$, $P < 0.001$) and the occurrence of lactation at conception ($F_{1,74} = 10.1$, $P = 0.002$). Specifically, when the previous infant was a male, the probability that it survived was lower than when it was a female and mothers were not usually lactating around conception (Table 2).

We recorded 49 births in high-quality fragments and 27 in low-quality fragments. In high-quality fragments the proportion of fruit in the diet of females around conception was significantly higher than in low-

quality fragments ($F_{1,74} = 6.3$, $P = 0.014$). When compared with high-quality fragments, in low-quality fragments there was a significantly lower proportion of births associated with lactation around conception ($F_{1,74} = 5.9$, $P = 0.017$), a lower proportion of previous infant survival ($F_{1,74} = 7.4$, $P = 0.008$), and a higher proportion of males born ($F_{1,74} = 5.6$, $P = 0.020$) (Table 3).

Predictors of sex at birth

From the 76 births that were analyzed 57 corresponded to male births, which represented 75% of all births. Variation in the sex of infants was significantly explained by our model ($Z_{4,76} = 31.4$, $P < 0.001$; percentage of cases correctly predicted = 84.2%), although only the occurrence of lactation around conception had a significant effect. Specifically, male births were significantly more likely when mothers were not lactating at conception (GLMM estimate = -3.22, $F = 18.7$, $P < 0.001$).

DISCUSSION

Maternal condition has been associated to offspring sex determination through a number of proximate mechanisms, including variation in uterine glucose levels, hormone levels, and sex biased embryo development (Krackow 1995; Cameron 2004; James 2008). We could not test these mechanisms in this study, but using indirect measures of maternal condition, we found support for the hypothesis that maternal condition around conception affects offspring sex determination in black howler monkeys. Our model indicated that the probability to conceive a male infant increased when females were not lactating around conception.

Lactation affects ovarian function. During lactation maternal energy is consistently diverted from reproduction to milk production, resulting on a negative en-

ergy balance which leads to lactational amenorrhea (Gittleman & Thompson 1988; Valeggia & Ellison 2004). Dependency of infants from maternal nursing correlates negatively with energy balance, such that energy balance shifts progressively towards a positive state as infants start complementing their diet with solid foods, allowing for the resumption of ovarian activity (Emery Thompson 2013a). The duration of lactational amenorrhea is highly variable both within and among individuals (e.g., humans: Wood 1994; chimpanzees, *Pan troglodytes*: Emery Thompson 2013b), but there is evidence that infant sex affects maternal investment in nursing. For instance, the nutritional value of milk (red deer: Landete-Castillejos *et al.* 2005; rhesus macaques: Hinde 2007; tammar wallaby: Robert & Braun 2012) and the duration of the lactation period (chimpanzees:

Table 2. Frequencies of previous infant survival and occurrence of lactation at conception according to the sex of the previous infant in black howler monkeys (N = 76 births) studied in Campeche (Mexico) between 2006 and 2012

		Sex of previous infant	
		Male	Female
Previous infant survived?	Yes	18	21
	No	33	4
Lactation at conception?	Yes	8	13
	No	43	12

Fahy *et al.* 2014) may vary between sexes. Such differential investment should affect maternal energy balance, the timing of ovarian function resumption, and maternal condition around conception. Based on sex differences in maturation and adult body size (Froehlich *et al.* 1981; Leigh 1994; Kitchen 2000; Kelaita *et al.* 2011), we assumed that the production of male black howler monkeys was costlier than that of females, and therefore the probabilities of conceiving a male should increase with increasing maternal condition. Our results support this prediction, as more male births occurred when mothers were not lactating around conception. However, high maternal condition around the conception of a male was related to the death of a previous son, rather than being associated with the conception and rearing of a female infant, and therefore, lower maternal investment in previous offspring as observed in other mammals (e.g., bighorn sheep: Berube *et al.* 1996; fallow deer, *Dama dama*: Birgersson 1998). Sixty five percent of male infants analyzed as a previous birth died, whereas only 16% of females did. Therefore, high mortality of sons, and the consequent interruption in maternal investment in nursing, seems to be the main determinant of better maternal condition around the conception of males in this population of black howler monkeys.

This putative effect of nursing effort around conception on sex determination mediated by previous infant sex and survival probability had not been reported before in non-human primates. Compared to other mammals, primates have longer lactation (Dufour & Sauter 2002), which is the costlier part of the reproductive process of primate females (Emery Thompson 2013b). It is therefore possible that lactation is a better predictor of maternal condition than other indirect measures of maternal condition for which a physiologi-

Table 3. Frequencies of sex and survival of previous infant, and occurrence of lactation at conception according to habitat quality in black howler monkeys (N = 76 births) studied in Campeche (Mexico) between 2006 and 2012

		Habitat quality	
		High	Low
Sex of previous infant	Male	28	23
	Female	21	4
Previous infant survived?	Yes	31	8
	No	18	19
Lactation at conception?	Yes	18	3
	No	31	24

cal significance on females has not been demonstrated, such as dominance rank in some primate species. For instance, the basis for dominance relationships in spider monkeys is not well understood (Strier 1999): agonistic interactions and spatial displacements among female spider monkeys (*Ateles* spp.) are infrequent, making the discrimination of dominance relationships difficult (Aureli & Schaffner 2008). Therefore, using female dominance rank as a predictor of maternal condition in spider monkeys (Symington 1987) may be problematic. We thus consider that the use of nursing effort around conception represents an interesting area for future non-invasive research on sex allocation in primates.

Body weight has been used in previous studies of sex allocation in mammals as a proxy for maternal condition (e.g., toque macaques: Dittus 1998; roe deer, *Capreolus capreolus*: Hewison *et al.* 1999; red deer: Luna-Estrada *et al.* 2006). Due to the non-invasive character of our study, we could not determine body weights of females around conception. However, during periods of fruit scarcity howler monkeys lose weight (Glander 2006; Espinosa-Gómez *et al.* 2013), so we expected the amount of consumed fruit to have a positive effect on maternal condition, and therefore to affect sex determination. Furthermore, because fruits are the main source of soluble sugars in the diet of howler monkeys (Espinosa-Gómez *et al.* 2013), variation in fruit consumption should covary with circulating levels of glucose, which have recently been demonstrated to affect sex determination in mammals (Ryan *et al.* 2012; Schwanz & Robert 2014). Fruit consumption around conception did not have, however, a significant effect on sex alloca-

tion in our model. This result is probably linked to an important degree of reproductive seasonality in this population, as approximately 70% of all births occur in the wet season (Dias *et al.* 2015). The clustering of births is linked to a strategy of offsetting the energetic demands of lactation by consuming more fruits (Dias *et al.* 2011). This suggests that, because females prioritize synchronizing lactation with high availability of fruits over conceiving during periods of fruit abundance, variation in the amount of ingested fruit around conception is low. Thus, the dietary patterns of female black howler monkeys around conception are a poor predictor of sex determination. Still, results on female feeding behavior should be interpreted with caution due to small sample size.

Previous research has demonstrated that spatial and temporal environmental heterogeneity is linked to variation in maternal condition (Pederson & Harper 1984; Dittus 1998; Banks *et al.* 2008). We thus predicted that, because females sampled in this study lived in highly heterogeneous environmental conditions, if maternal condition determined offspring sex, sex ratios at birth should vary among fragments. Contrary to this prediction fragment quality had a non-significant effect on sex determination. Because low-quality fragment were associated with more male births, lower infant survival and fewer females lactating around conception, it is possible nevertheless that the above mentioned interplay of infant sex, infant survival and maternal condition is influenced by habitat quality, and that the lack of a direct effect of this variable on sex determination resulted from low statistical power (e.g., only 27 births were recorded in low-quality habitats, only four daughters were analyzed as a previous birth). Furthermore, we have previously suggested that the mortality of infant black howler monkeys is higher in low-quality fragments due to a reduction in nursing quality of mothers caused by low access to food resources (Dias *et al.* 2015). These results raise the possibility that the observed patterns of sex determination derive from a mismatch between a mechanism that evolved for allowing females in good condition to skew offspring sex ratios in favor of the sex that provided higher fitness returns (i.e., sons; Trivers & Willard 1973) and adverse contemporary ecological conditions that lead to a feedback between male mortality and maternal condition, as explained above. The fact that black howler monkeys living in disturbed habitats have higher fecal glucocorticoid levels (Rangel-

Negrín *et al.* 2014) could additionally account for male skewed sex ratios at birth if variation in these hormones is proximally associated with sex determination, as observed in other species (Ryan *et al.* 2012). Disentangling current environmental heterogeneity of physiological mechanisms for sex determination and selective pressures that have led to them is a considerable challenge for future research of sex allocation in black howler monkeys and other mammals. Non-invasively measuring variation in the metabolic status of females throughout the reproductive process will be our next step. In any case, these patterns raise the possibility that the viability of populations living in disturbed habitats is decreasing due to higher infant mortality.

In sum, we found evidence that maternal condition affects sex determination in black howler monkeys: the occurrence of lactation around the time of conception increased the probabilities of conceiving daughters. However, this effect was probably determined by a positive relationship between male infant mortality and the conception of sons: females that were not lactating around the time of conception produced more sons. We speculate that this relationship allowed females to be released of the energetic burden of nursing when a new infant was conceived. Future research on the physiological mechanisms underlying these findings should allow understanding the adaptive significance of sex allocation in black howler monkeys, which cannot currently be assessed.

ACKNOWLEDGEMENTS

Fifty three people were involved in fieldwork; we thank all of them for their invaluable efforts. The following people and institutions granted permission to work in their properties and facilitated our fieldwork: Comisarios Ejidales de Abelardo Domínguez, Calax, Chekubul, Conhuas, Nvo. Ontario, Plan de Ayala, and Candelario Hernández Perera, Igor, Carmén Gómez and Ricardo Valencia; Ayuntamiento de Calakmul; M.C. Mario Rivera de Labra and Ing. A. Sánchez Martínez, El Tormento, INIFAP; Lic. C. Vidal and Lic. L. Álvarez, INAH Campeche; Biól. F. Durand Siller, Reserva de la Biósfera Calakmul, CONANP; Ing. V. Olvera, El Álamo. Climate data was kindly provided by CONAGUA. We thank the help of R. Mateo-Gutiérrez, S. Sinaca-Colín, C. Gutiérrez-Báez (Centro de Investigaciones Históricas y Sociales, UACM) and J. Sal-

vador Flores-Guido (Facultad de Medicina Veterinaria y Zootecnia, UADY) in the identification of plant species. This study was supported by Comisión Federal de Electricidad (RGCP-TP-UV-001/04), Universidad Veracruzana, Consejo Nacional de Ciencia y Tecnología (grant number: 235839; grants i010/152/2014 & C-133/2014) and Idea Wild. We are indebted to V. Arroyo-Rodríguez and W. Dáttilo for support with statistical analysis, and to F. Aureli, R.G. Teixeira da Cunha and two anonymous reviewers for helpful comments and suggestions that greatly improved the manuscript. We thank the editors for the invitation to write this chapter. P.A.D.D. and A.R-N. thank Mariana and Fernando for being a constant source of inspiration for exploring primate behavior and ecology.

BIBLIOGRAPHY

- Arroyo-Rodríguez V & PAD Dias (2010) Effects of habitat fragmentation and disturbance on howler monkeys: a review. *American Journal of Primatology* 71:1-16.
- Arroyo-Rodríguez V, González-Pérez IM, Garmendia A, Solà M & A Estrada (2013) The relative impact of forest patch and landscape attributes on black howler monkey populations in the fragmented Lacandona rainforest, Mexico. *Landscape Ecology* 28:1717-1727.
- Aureli F & CM Schaffner (2008) Social interactions, social relationships and the social system of spider monkeys. In: Campbell CJ (ed.) *Spider Monkeys: The Biology, Behavior and Ecology of the Genus Ateles*. Cambridge University Press. Cambridge, UK. Pp. 236-265.
- Banks SC, Knight EJ, Dubach JE & DB Lindenmayer (2008) Microhabitat heterogeneity influences offspring sex allocation and spatial kin structure in possums. *Journal of Animal Ecology* 77:1250-1256.
- Behie AM & MSM Pavelka (2015) Fruit as a key factor in howler monkey population density: conservation implications. In: Kowalewski M, Garber PA, Cortés-Ortiz L, Urbani B & D Youlatos (eds.) *Howler Monkeys: Behavior, Ecology and Conservation*. Springer. New York, USA. Pp. 357-382.
- Berman CM (1988) Maternal condition and offspring sex ratio in a group of free-ranging rhesus monkeys: an 11-year study. *American Naturalist* 131:307-328.
- Berube CH, Festa-Bianchet M & JT Jorgenson (1996) Reproductive costs of sons and daughters in rocky mountain big-horn sheep. *Behavioral Ecology* 7:60-68.
- Birgersson B (1998) Adaptive adjustment of the sex ratio: more data and considerations from a fallow deer population. *Behavioral Ecology* 9:404-408.
- Bonier F, Martin PR & JC Wingfield (2007) Maternal corticosteroids influence primary offspring sex ratio in a free-ranging passerine bird. *Behavioral Ecology* 18:1045-1050.
- Cameron EZ (2004) Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. *Proceedings of the Royal Society of London B* 271:1723-1728.
- Canales-Espinosa D, Rovirosa-Hernández MJ, de Thoisy B, Caba-Vinagre M & F García-Orduña (2015) Hematology and serum biochemistry in wild howler monkeys. In: Kowalewski M, Garber PA, Cortés-Ortiz L, Urbani B & D Youlatos (eds.) *Howler Monkeys: Adaptive Radiation, Systematics, and Morphology*. Springer. New York, USA. Pp. 179-202.
- Clark AB (1978) Sex ratio and local resource competition in a prosimian primate. *Science* 201:163-165.
- Clutton-Brock TH & GR Iason (1986) Sex ratio variation in mammals. *Quarterly Review of Biology* 61:339-374.
- Dias PAD, Alvarado D, Rangel-Negrín A, Canales-Espinosa D & L Cortés-Ortiz (2013) Landscape attributes affecting the natural hybridization of Mexican howler monkeys. In: Marsh L & CA Chapman (eds.) *Primates in Fragments: Complexity and Resilience*. Springer. New York, USA. Pp. 423-435.
- Dias PAD, Coyohua-Fuentes A, Canales-Espinosa D & A Rangel-Negrín (2015) Group structure and dynamics in black howler monkeys: a 7-year perspective. *International Journal of Primatology* 36:311-331.
- Dias PAD, Coyohua-Fuentes A, Canales-Espinosa D & A Rangel-Negrín (2016) Factors influencing the reproductive success of female black howler monkeys (*Alouatta pigra*). *International Journal of Primatology* 37:638-655.
- Dias PAD, Rangel-Negrín A & D Canales-Espinosa (2011) Effects of lactation on the time-budgets and foraging patterns of female black howlers (*Alouatta pigra*). *American Journal of Physical Anthropology* 145:137-146.
- Dias PAD, Rangel-Negrín A, Coyohua-Fuentes A & D Canales-Espinosa (2014) The dietary breadth of a Neotropical primate is not associated with vegetation disturbance. *American Journal of Primatology* 76:1151-1162.
- Dittus WPJ (1998) Birth sex ratios in toque macaques and other mammals: integrating the effects of maternal condition and competition. *Behavioral Ecology Sociobiology* 4:149-160.
- Dufour DJ & ML Sauter (2002) Comparative and evolutionary dimensions of the energetics of human pregnancy and lactation. *American Journal of Human Biology* 14:584-602.
- Emery Thompson M (2013a) Reproductive ecology of wild chimpanzees. *American Journal Primatology* 75:222-237.
- Emery Thompson M (2013b) Comparative reproductive energetics of human and non-human primates. *Annual Review of Anthropology* 42:287-304.
- Espinosa-Gómez F, Gómez-Rosales S, Wallis IR, Canales-Espinosa D & L Hernández-Salazar (2013) Digestive strategies and food choice in mantled howler monkeys *Alouatta palliata mexicana*: bases of their dietary flexibility. *Journal of Comparative Physiology B* 183:1089-1100.
- Fahy GE, Richards MP, Fuller BT, Deschner T, Hublin JJ & C Boesch (2014) Stable nitrogen isotope analysis of dentine serial sections elucidate sex differences in weaning patterns of wild chimpanzees (*Pan troglodytes*). *American Journal of Physical Anthropology* 153:635-642.

- Froehlich JW, Thorington RW & JS Otis (1981) The demography of howler monkeys (*Alouatta palliata*) on Barro Colorado Island, Panamá. *International Journal of Primatology* 2:207-236.
- Gittleman JL & SD Thompson (1988) Energy allocation in mammalian reproduction. *American Zoologist* 28:863-875.
- Glander KE (2006) Average body weight for mantled howling monkeys (*Alouatta palliata*): an assessment of average values and variability. In: Estrada A, Garber PA, Pavelka MSM & L Luecke (eds.) *New Perspectives in the Study of Mesoamerican Primates: Distribution, Ecology, Behavior, and Conservation*. Springer. New York, USA. Pp. 247-263.
- Gomendio M, Clutton-Brock TH, Albon SD, Guinness FE & MJ Simpson (1990) Mammalian sex ratios and variation in costs of rearing sons and daughters. *Nature* 343:261-263.
- Hendrickx J (2015) Package 'perturb'. Available at <http://cran.rproject.org/web/packages/perturb/perturb.pdf> (Accessed March 15-X-2016).
- Hewison AJM, Andersen M, Gaillard J-M, Linnell JDC & D Delorme (1999) Contradictory findings in studies of sex ratio variation in roe deer (*Capreolus capreolus*). *Behavioral Ecology and Sociobiology* 45:339-348.
- Hinde K (2007) First time macaque mothers bias milk composition in favor of sons. *Current Biology* 17:958-959.
- Hogg JC, Hass CC & DA Jenni (1992) Sex-biased maternal expenditure in Rocky Mountain bighorn sheep. *Behavioral Ecology and Sociobiology* 31:243-251.
- James WH (2008) Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels around the time of conception. *Journal of Endocrinology* 198:3-15.
- Kelaita M, Dias PAD, Aguilar-Cucurachi M, Canales-Espinosa D & L Cortés-Ortiz (2011) Impact of intrasexual selection on sexual dimorphism and testes size in the Mexican howler monkeys *Alouatta palliata* and *A. pigra*. *American Journal of Physical Anthropology* 146:179-187.
- Kitchen DM (2000) *Aggression and assessment among social groups of Belizean black howler monkeys (Alouatta pigra)*. Ph. D. thesis. University of Minnesota. USA. 286 pp.
- Krackow S (1995) Potential mechanisms for sex ratio adjustment in mammals and birds. *Biological Reviews* 70:225-241.
- Kruuk LEB, Clutton-Brock TH, Albon SD, Pemberton JM & FE Guinness (1999) Population density affects sex ratio variation in red deer. *Nature* 399:459-461.
- Landete-Castillejos T, García A, López-Serrano FR & L Gallego (2005) Maternal quality and differences in milk production and composition for male and female Iberian red deer calves (*Cervus elaphus hispanicus*). *Behavioral Ecology and Sociobiology* 57:267-274.
- Law PR, Fike B & PC Lent (2014) Birth sex in an expanding black rhinoceros (*Diceros bicornis minor*) population. *Journal of Mammalogy* 95:349-356.
- Leigh SR (1994) Ontogenetic correlates of diet in anthropoid primates. *American Journal of Physical Anthropology* 94:499-522.
- Love OP, Chin EH, Wynne-Edwards KE & TD Williams (2005) Stress hormones: a link between maternal condition and sex-biased reproductive investment. *American Naturalist* 166:751-766.
- Luna-Estrada AA, Vera-Avila HR, Mora O, Anguiano-Serrano B, Vasquez-Pelaez CG & A Shimada (2006) Effect of pre-mating nutritional status in red deer (*Cervus elaphus scoticus*) hinds on the sex ratio of their offspring. *Small Ruminant Research* 65:154-160.
- Martin JGA & M Festa-Bianchet (2011) Sex ratio bias and reproductive strategies: what sex to produce when? *Ecology* 92:441-449.
- Packer C, Collins DA & LE Eberly (2000) Problems with primate sex ratios. *Proceedings of the Royal Society of London B* 355:1627-1635.
- Pederson JC & KT Harper (1984) Does summer range quality influence sex ratios among mule deer fawns in Utah? *Journal of Range Management* 37:64-66.
- R Development Core Team (2016) R: a language and environment for statistical computing. <http://www.R-project.org>. R Foundation for Statistical Computing, Vienna, Austria
- Rabe-Hesketh S, Skrondal A & A Pickles (2005) Maximum likelihood estimation of limited and discrete dependent variable models with nested random effects. *Journal of Econometrics* 128:301-323.
- Rangel-Negrín A, Coyohua-Fuentes A, Chavira R, Canales-Espinosa D & PAD Dias (2014) Primates living outside protected habitats are more stressed: the case of black howler monkeys in the Yucatán Peninsula. *PLOS One* 9:e112329.
- Rijt-Plooij HHC & FX Plooij (1987) Growing independence, conflict and learning in mother-infant relations in free-ranging chimpanzees. *Behaviour* 101:1-86.
- Robert KA & S Braun (2012) Milk composition during lactation suggests a mechanism for male biased allocation of maternal resources in the tammar wallaby (*Macropus eugenii*). *PLOS One* 7:e51099.
- Rutberg AT (1986) Lactation and the foetal sex ratios in American bison. *American Naturalist* 127:89-94.
- Ryan CP, AndersonWG, Gardiner LE & JF Hare (2012) Stress-induced sex ratios in ground squirrels: support for a mechanistic hypothesis. *Behavioral Ecology* 23:160-167.
- Saltz D (2001) Progeny sex ratio variation in ungulates: maternal age meets environmental perturbation of demography. *Oikos* 94:377-384.
- Schaffner CM, Rebecchini L, Aureli F, Ramos-Fernandez G & L Vick (2012) Spider monkeys cope with the negative consequences of hurricanes through changes in diet, activity budget and fission-fusion dynamics. *International Journal of Primatology* 33:922-936.
- Schino G (2004) Birth sex ratio and social rank: consistency and variability within and between primate groups. *Behavioral Ecology* 15:850-856.
- Schwanz LE & KA Robert (2014) Proximate and ultimate explanations of mammalian sex allocation in a marsupial model. *Behavioral Ecology and Sociobiology* 68:1085-1096.

- Silk JB (1983) Local resource competition and facultative adjustment of sex ratios in relation to competitive abilities. *American Naturalist* 121:55-66.
- Silk JB & GR Brown (2004) Sex ratios in primate groups. In: Kappeler PM & CP van Schaik (eds.) *Sexual Selection in Primates: New and Comparative Perspectives*. Cambridge University Press. Cambridge, UK Pp. 253-265.
- Silk JB & SC Strum (2007) Maternal condition does not influence birth sex ratios in Anubis baboons (*Papio anubis*). *PLOS One* 5:e12750.
- Silk JB, Willoughby E & GR Brown (2005) Maternal rank and local resource competition do not predict birth sex ratios in wild baboons. *Proceedings of the Royal Society of London B* 272:569-574.
- Strier KB (1999) Why is female kin bonding so rare? Comparative sociality of Neotropical primates. In: Lee PC (ed.) *Comparative Primate Socioecology*. Cambridge University Press. Cambridge. Pp. 300-319.
- Symington MM (1987) Sex ratio and maternal rank in wild spider monkeys: when daughters disperse. *Behavioral Ecology and Sociobiology* 20:421-425.
- Trivers RL & D Willard (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90-92.
- Valeggia CR & PT Ellison (2004) Lactational amenorrhoea in well-nourished Toba women of Formosa, Argentina. *Journal of Biosocial Science* 36:573-595.
- Van Belle S, Estrada A & KB Strier (2011) Insights into social relationships among female black howler monkeys *Alouatta pigra* at Palenque National Park, Mexico. *Current Zoology* 57:1-7.
- Van Belle S, Estrada A & KB Strier (2008) Social relationships among male *Alouatta pigra*. *International Journal of Primatology* 29:1481-1498.
- Van Belle S, Estrada A, Ziegler TE & KB Strier (2009a) Social and hormonal mechanisms underlying male reproductive strategies in black howler monkeys (*Alouatta pigra*). *Hormones and Behavior* 56:355-363.
- Van Belle S, Estrada A, Ziegler TE & KB Strier (2009b) Sexual behavior across ovarian cycles in wild black howler monkeys (*Alouatta pigra*): male mate guarding and female mate choice. *American Journal of Primatology* 71:153-164.
- van Schaik CP & SB Hrdy (1991) Intensity of local resource competition shapes the relationship between maternal rank and sex ratios at birth in cercopithecine primates. *American Naturalist* 138:1555-1562.
- West SA & BC Sheldon (2002) Constraints in the evolution of sex ratio adjustment. *Science* 295:1685-1688.
- Wood JW (1994) *Dynamics of Human Reproduction: Biology, Biometry, Demography*. Aldine de Gruyter. New York, USA. 669 pp.