Primates
https://doi.org/10.1007/s10329-020-00883-6

ORIGINAL ARTICLE

Maternal care according to offspring sex and maternal physical condition in mantled howler monkeys (*Alouatta palliata*)

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Received: 13 July 2020 / Accepted: 9 December 2020
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Abstract
The Trivers–Willard hypothesis (TWH) posits that maternal care will be biased in favor of the sex that provides the greatest fitness returns per unit of investment, depending on maternal physical condition. Our aim was to examine the TWH in mantled howler monkeys living at Los Tuxtlas (Veracruz, Mexico). The biological attributes of mantled howler monkeys (*Alouatta palliata*) meet the assumptions of TWH better than those of other explanations, so we expected that females in better physical condition should bias maternal care toward sons, whereas mothers in worse physical condition should bias care toward daughters. Between December 2017 and March 2019, we studied mother–infant interactions in 20 dyads with focal-animal sampling and continuous recording ($N = 204$ h). We performed genetic analysis to determine offspring sex ($N = 7$ daughters and 13 sons) and measured C-peptide in urine samples of mothers to assess their physical condition ($N = 46$ samples). Mothers in better physical condition spent less time in contact with their sons but more time in contact with their daughters. For proximity behavior, mothers in better physical condition spent more time near their sons and less time near their daughters. These results suggest a bias in maternal care towards daughters, contrary to our predictions. In light of current models of maternal investment, our results support that mothers obtain higher fitness returns through daughters.

Keywords C-peptide · Maternal investment · Offspring sex · Parental care · Physical condition

Introduction

The goal of sex allocation theory is to predict the optimal allocation of investment of a parent to male and female offspring under diverse environmental and social conditions (Charnov 1982; De Jong and Klinkhamer 2005). Fisher (1930) argued that when the cost of production is the same for male and female offspring, natural selection is expected to maintain an equal ratio of males and females at birth. When the cost of progeny varies according to sex, the sex ratio at birth will be biased towards the sex that requires less investment (Fisher 1930). Since Fisher, many studies have reported biases in the sex ratio of offspring in various organisms, and three main hypotheses have been proposed to explain these biases.

First, Hamilton (1967) developed the local mate competition hypothesis, arguing that a bias in offspring sex ratio may occur when siblings compete for reproduction. In fig wasps (*Blasophaga psenes*), it has been observed that in a context of intense local mate competition, mothers only produce the number of males required to fertilize all their female offspring (Silk and Brown 2004). Second, in species in which one of the sexes disperses, individuals of the philopatric sex will compete against relatives when resources are locally limited (Clark 1978). In this situation, local resource competition will favor females that produce more offspring of the dispersing sex. Third, the Trivers and Willard (1973) hypothesis (TWH hereafter) states that differences in variance of reproductive success between sons and daughters generate a bias in maternal investment depending on

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Published online: 01 February 2021

maternal condition. Sex biases in maternal investment may be manifested either in birth sex ratio, in maternal expenditure during pre- and postnatal life (i.e., maternal investment), or both (Cameron and Linklater 2002; Hewison and Gaillard 1999). This hypothesis is based on the following theoretical arguments: (1) offspring condition at the end of the parental investment period correlates with the condition of the mother; (2) differences in condition among offspring at the end of the parental investment period will endure into adulthood; and (3) early development has greater impact on the reproductive success of one sex than on the other (Trivers and Willard 1973).

Mothers in good condition are predicted to bias their investment in favor of the sex that will provide the greatest fitness returns measured by the number of grand-offspring per unit of investment (Leimar 1996; Maynard Smith 1980; Reiter et al. 1978; Trivers and Willard 1973). In mammals, variance in reproductive success is usually greater in males than in females, especially in polygynous species (Clutton-Brock et al. 1977, 1979; Le Boeuf 1974; Trivers 1972). Thus, the TWH predicts that in polygynous and sexually dimorphic species, females should invest more heavily in sons than in daughters when they are in good condition. Theoretical models suggest that this prediction may not always hold (Borgstede 2019; Leimar 1996; Veller 2016). Leimar (1996), for instance, proposed that when maternal condition is transmitted with better fidelity to daughters than to sons, females in good condition will benefit more from investment in daughters than sons. Given that the social, ecological, and demographic context of populations could change fitness returns provided by each offspring according to their sex (Altmann and Altmann 1991; Perret 1990, 1996), there is not a clear pattern of investment that mammalian mothers should follow.

Parental investment is the energy or resources that a progenitor allocates to increase the probability of survival and reproduction of its current offspring (Trivers 1972). Investment in current offspring will result in a reduction in the parents' ability to invest in future offspring. Thus, investment in offspring is a trade-off (Trivers 1972). Empirical calculation of the direct benefits offspring receive from their parents and of the costs of investment to parents has been an elusive task, hindering the assessment of the TWH (Brown 2001). To test sex-biased maternal investment, most studies have relied on measures of maternal care (e.g., nursing time, transport time) without considering the costs of these behaviors in terms of energy or fitness (Brown 2001; Clutton-Brock 1991; Remick 1992). It is known that maternal care is highly dependent on the physical condition of mothers (Fairbanks and Hinde 2013), resulting in an inverted U-shaped relationship between the maternal physical condition and the amount of care provided to the offspring (Fairbanks and McGuire 1995). For example, in vervet monkeys (*Chlorocebus pygerythrus*), mothers in bad and good condition provide less maternal care (i.e., less time in contact and more rejection) than mothers in average condition. For mothers in bad condition, limiting the investment protects their own health even at the cost of higher infant mortality, whereas for mothers in good condition it shortens interbirth intervals without jeopardizing the survival of infants (Fairbanks and Hinde 2013).

The TWH has been evaluated in several species by analyzing the influence of maternal condition on either birth sex ratios or maternal investment during pre- and postnatal periods (Brown 2001). Few studies have provided conclusive results, and these are often inconsistent, both within and between species (Brown 2001; Cameron 2004). Such inconsistencies could result from several factors, including variation in methods for assessing maternal condition, interspecies differences in investment strategies, or variation in maternal strategies due differences in demographic, social, and ecological contexts (Altmann and Altmann 1991; Perret 1990, 1996; Schino et al. 1995; Silk and Brown 2004). Additionally, most research has focused on birth sex ratios, with very few studies examining the prediction of the hypothesis concerning post-partum investment (Douhard 2017). Determining how females distribute care between offspring of different sex is crucial for understanding the evolution of reproductive strategies and predicting variance in reproductive success (Young and Badyaev 2004).

The biological attributes of mantled howler monkeys (*Alouatta palliata*) meet the assumptions of the TWH better than those of other explanations. First, males have higher mortality but can potentially produce more offspring than females, suggesting that variance in reproductive success should be higher in males than in females (Clarke and Glander 1984). In a Costa Rican population, an average female produces approximately eight offspring during her lifetime, while a male can produce between zero and 18 offspring (Clarke and Glander 1984). Second, given the faster growth rates during the first year of life and delayed onset of solid food consumption of sons compared to daughters (Raguet-Schofield and Pavé 2015), sons should be energetically costlier than daughters. Third, both sexes commonly disperse from natal groups (Cristóbal-Azkarate et al. 2017). Therefore, the aim of the present study was to analyze whether the maternal care patterns of mantled howler monkeys correspond to those expected under the TWH. Accordingly, we predicted that mothers in better physical condition should bias their care towards sons, whereas mothers in worse physical condition should bias their care towards daughters.

Methods

Study site and subjects

The study was conducted from December 2017 to March 2019 at the Los Tuxtlas region (southeast Veracruz, Mexico). The region was originally covered by tropical evergreen forest, but has been severely deforested over the last decades, resulting in a mosaic landscape of forest fragments surrounded by a matrix of pasturelands, croplands, and urban areas (Arroyo-Rodríguez et al. 2011; Castillo-Campos and Laborde 2004). Ambient temperature fluctuates between 24 °C and 26 °C, and rainfall is ca. 3000–4000 mm, with most rain concentrated between June and February, i.e., the rainy season (Soto and Gama 1997).

We worked in five forest fragments: Cerro del Borrego (18°38′32″ N 95°05′30″ W), Montepío 2 (18°37′12″ N 95°04′57″ W), Ripario (18°36′33″ N 95°04′16″W), Balzapote (18°36′38″ N 95°04′11″ W), and La Flor de Catemaco (18°26′17″ N 95°03′01″ W). Mean ± SD group size was 19.7 ± 13.4 individuals (range = 5–39), with 5.5 ± 2.5 adult males (range = 2–9), 8.1 ± 6.1 adult females (range = 2–16), 2.7 ± 1.5 juveniles (range = 1–5), and 6.5 ± 5.4 infants (range = 1–15). We classified infants into three age categories: infants 1 (0 to < 4 months), infants 2 (4 to < 9 months), and infants 3 (9 to < 16 months; Balcells and Veà 2009). For two dyads, sampling spanned more than one infant age category. We used anatomical and physiognomic characteristics for the individual identification of adult females and infants, including body size and proportions, scars, broken fingers, and genital morphology, as well as blond hairs and skin pigmentation on feet, hands, and tail.

Behavioral observations

We used focal-animal sampling with continuous recording (8-h samples; Altmann 1974) to study the duration of maternal care behaviors (Table 1). During the 16 study months, we obtained 300 observation hours of mother–offspring dyads for which we could determine infant sex and maternal physical condition (see below), with a mean ± SD of 10.7 ± 6.7 h (2–29.2) per female per infant category (Table 2).

Table 2 Number of observation hours per mother–infant dyad according to the age category of infants in *Alouatta palliata* females studied at Los Tuxtlas (Mexico)

Mother	Infant sex	Age		
		Infant 1	Infant 2	Infant 3
1	Female	6.5	–	–
2	Male	18.0	–	–
3[a]	Male	17.5	–	–
4	Male	29.2	–	–
5	Male	14.0	–	–
6	Male	7.0	–	–
7	Male	15.0	19.0	–
8	Male	–	14.7	5.0
9	Male	–	9.2	–
10	Male	–	6.0	–
11[a]	Female	–	3.5	–
12	Male	–	8.0	–
13	Male	–	7.0	–
14	Female	–	7.1	–
15	Male	–	16.0	8.0
16	Female	–	2.0	–
17	Male	–	6.0	–
18	Male	–	–	7.3
19	Male	–	–	26.0
20	Male	–	–	7.0
21	Female	–	–	14.2
22	Female	–	–	7.0
23	Male	–	–	4.2
24	Male	–	–	10.3
25	Female	–	–	5.4
Total		107.2	98.5	94.4

[a]The only female that was sampled for more than one offspring

Table 1 Maternal care behaviors of *Alouatta palliata* mothers assessed in this study conducted at Los Tuxtlas (Mexico)

Behavior	Definition	References
Ventro-ventral contact	Mother and infant had their bellies in contact	Schino et al. 1995
Dorso-ventral contact	Mother's back in contact with the infant's belly	Ross 2001
Generic contact	Contact between mother and infant different from ventro-ventral and dorso-ventral contact	Brown and Dixson 2000
Proximity	Mother and infant were < 1 m away	Brown and Dixson 2000
Maternal Vigilance	Visual search of the environment beyond the immediate neighborhood; any exploration directed beyond the reach of the animal's arm	Treves 2000

Assessment of female physical condition

To assess maternal physical condition, we determined the median urinary C-peptide concentrations of each mother, which has been validated as a measure of female energetic balance in primates (Emery Thompson 2017), including mantled howler monkeys (Cano-Huertes et al. 2017; Dias et al. 2018). We collected urine samples from plastic sheets positioned under females or by collecting urine directly from the vegetation. Urine contaminated by feces, soil, water, or urine from another individual was not collected (Emery Thompson and Knott 2008). We kept urine samples cooled in a thermo cooler with ice packs and froze them at −20 °C within a maximum of 12 h after collection. The assessment of urinary C-peptide concentrations was performed at the "Laboratorio de Hormonas Esteroides" of the "Instituto Nacional de Ciencias Médicas y Nutrición". We quantified C-peptide concentrations via enzyme immunoassay following the manufacturer's instructions (C-Peptide ELISA, DRG International, Inc., USA). We standardized C-peptide measurements by dividing C-peptide concentrations by creatinine concentrations (Cano-Huertes et al. 2017), which we measured in each sample via enzyme immunoassays with an auto-analyzer (Gallery, Thermo Fisher Scientific, Waltham, MA, USA). Serial dilutions of samples did not differ from their respective standard curves ($F_4 = 0.52$, $P > 0.05$). The inter-assay coefficient of variation (CV) of controls for the C-peptide assay was 8.8% ($N = 3$), and intra-assay CV was 9.1% ($N = 3$). We collected a total of 139 urine samples, with a mean ± SD number of 1.9 ± 3.5 samples collected per female. We found no significant differences in C-peptide concentrations between samples collected in the morning and in the afternoon (linear model $\chi^2_{139} = 2.45$, $P = 0.352$).

Fecal sample collection and offspring sex determination

Given that sex in mantled howler monkey infants cannot be visually determined in the field (Clarke et al. 2007; Dias et al. 2020), we performed genetic sex determination in fecal samples. We collected fecal samples opportunistically: each time an immature was observed defecating, we collected its feces immediately from the forest floor and deposited it in a polyethylene bag labeled with the identity of the individual. We kept feces in a cooler with frozen gel packs while in the field. At the end of the day, samples were stored in a freezer at −20 °C until DNA extraction was performed. DNA was extracted following a modification of the methods described in Kanai et al. (1994), and sex assignment was performed following the procedures described in Di Fiore (2005; Supporting information).

Data analysis

We organized data per mother–infant dyad for each infant age category. Accordingly, we summed the duration of each behavior category across all focal samples of each dyad per infant age category and calculated median urinary C-peptide concentration values for each mother per infant age category.

To analyze the influence of offspring sex and maternal physical condition on the duration of each maternal care behavior, we used generalized linear mixed models with beta-binomial distribution to handle the overdispersion in our binomial data. Maternal care behaviors were analyzed as two-vector dependent variables composed of time engaged in a given care behavior and time not engaged in that behavior. Fixed predictive factors were offspring sex, median maternal physical condition (urinary C-peptide concentration), and their interaction. As maternal physical condition was collinear with infant age because of lower C-peptide concentrations in mothers of infants 1, we only analyzed dyads including infants 2 and infants 3, whose mothers do not show significant variation in C-peptide concentrations (Dias et al. 2018). After removing mother–infant 1 dyads, our data consisted of 20 mother–offspring dyads. According to the results of genetic analysis, our sample included seven daughters and 13 sons. Therefore, we analyzed 204 observation hours (9.1 ± 5.1 h per female per infant age category) and 46 urine samples (2.3 ± 3 samples per female per infant age category). Given that maternal behavior should vary with infant age (Dias et al. 2018), we also added the interaction between age category and offspring sex to models as a fixed factor to control for differences in the allocation of care between both ages. We added dyad identity as a random predictive factor to account for the repeated sampling of some dyads in more than one infant age category. To determine the influence of our predictive factors, for each behavior, we compared complete models (i.e., including both fixed and random predictors) with models including only the random factor with likelihood ratio tests (LRT). A nonsignificant test result indicates that the random predictor had a stronger influence on behavioral variables than fixed predictors. Besides checking for multicollinearity among predictors with a variance inflation factor (VIF), we diagnosed residual distribution with Q-Q plots, overdispersion, and zero inflation and found that all models met statistical assumptions. We calculated Ω^2 to assess model goodness of fit (Xu 2003). All analyses were performed in R 4.0.1 (R core Team 2019), and we set alpha at 0.05 to assess significance.

Results

For the behavior "vigilance", the complete model was not different from the null model that included only the random factor (LRT vigilance $\chi^2_3 = 9.8$, $P = 0.082$). For the behavior

"dorso-ventral contact", the complete model was significantly different from the null model (LRT dorso-ventral contact $\chi^2_3 = 11.4$, $P = 0.04354$), but no fixed factor showed a significant effect on the behavior (Table 3).

The complete model for "ventro-ventral contact" was significantly different from the null model (LRT ventro-ventral contact $\chi^2_3 = 15.7$, $P = 0.0078$). The time mothers dedicated to ventro-ventral contact was affected by the interaction between offspring sex and infant age but not by offspring sex, maternal physical condition, or by the interaction between offspring sex and maternal physical condition (Table 4). Time in ventro-ventral contact was higher for

Table 3 Generalized linear mixed model results of maternal care behaviors that were not influenced by offspring sex, age, or maternal physical condition in mother–offspring mantled howler monkey (*Alouatta palliata*) dyads studied at Los Tuxtlas (Mexico)

Model/predictor[a]	B	SE	*z*	*P*	95% CI	
					Lower	Upper
Dorso-ventral contact $\Omega^2 = 0.63$						
Intercept	−1.9	0.9	−2.2	0.027	−3.574	−0.214
Offspring sex	−0.1	0.8	−0.1	0.885	−1.759	1.516
Maternal physical condition	−0.3	1.1	−0.3	0.797	−2.447	1.878
Offspring sex × maternal physical condition	−0.4	1.2	−0.4	0.718	−2.730	1.889
Offspring sex (f) × infant age (infant 3)	0.1	0.7	0.1	0.889	−1.318	1.521
Offspring sex (m) × infant age (infant 3)	−0.8	0.7	−1.1	0.239	−2.242	0.559
Maternal vigilance $\Omega^2 = 0.58$						
Intercept	−3.4	0.6	−5.9	< 0.001	−4.499	−2.242
Offspring sex	−0.2	0.6	−0.2	0.813	−1.401	1.099
Maternal physical condition	−0.4	0.7	−0.6	0.579	−1.855	1.036
Offspring sex × maternal physical condition	0.4	0.8	0.5	0.608	−1.166	1.991
Offspring sex (f) × infant age (infant 3)	0.5	0.6	0.8	0.422	−0.676	1.616
Offspring sex (m) × infant age (infant 3)	−1.1	0.6	−1.8	0.075	−2.259	0.107

[a]Goodness of fit (Ω^2) of each model indicated after each behavior name

Table 4 Generalized linear mixed model results of maternal care behaviors that were influenced by offspring sex, age, and maternal physical condition in mother–offspring mantled howler monkey (*Alouatta palliata*) dyads studied at Los Tuxtlas (Mexico)

Model/predictor[a]	β	SE	*z*	*P*	95% CI	
					Lower	Upper
Ventro-ventral contact $\Omega^2 = 0.57$						
Intercept	−1.1	0.6	−2	0.048	−2.247	−0.009
Offspring sex	− 0.6	0.6	−1.1	0.289	−1.871	0.557
Maternal physical condition	0.5	0.9	0.5	0.630	−1.460	2.412
Offspring sex × maternal physical condition	−0.9	1	−0.9	0.358	−2.985	1.080
Offspring sex (f) × infant age (infant 3)	−2.2	0.8	−2.8	0.005	−3.822	−0.648
Offspring sex (m) × infant age (infant 3)	−1.5	0.5	−2.7	0.006	−2.496	−0.404
Contact $\Omega^2 = 0.71$						
Intercept	−1.4	0.7	−2.1	0.039	−2.723	−0.067
Offspring sex	−0.3	0.7	−0.3	0.732	−1.669	1.173
Maternal physical condition	3.1	1.1	2.7	0.006	0.843	5.287
Offspring sex × maternal physical condition	−3.2	1.2	−2.8	0.006	−5.450	−0.933
Offspring sex (f) × infant age (infant 3)	−0.5	0.8	−0.6	0.541	−2.169	1.139
Offspring sex (m) × infant age (infant 3)	0.5	0.4	1.1	0.281	−0.387	1.331
Proximity $\Omega^2 = 0.89$						
Intercept	−12	0.4	−2.8	0.005	−2.025	−0.342
Offspring sex	0.7	0.5	1.7	0.082	1.701	0.799
Maternal physical condition	−1.2	0.7	−1.9	0.060	−2.508	0.052
Offspring sex × maternal physical condition	1.4	0.7	2.1	0.037	0.081	2.688
Offspring sex (f) × infant age (infant 3)	−0.6	0.4	−1.4	0.152	−1.329	0.207
Offspring sex (m) × infant age (infant 3)	−0.3	0.3	−1.2	0.246	−0.865	0.221

[a]Goodness of fit (Ω^2) of each model indicated after each behavior name

mother–infant 2 than for mother–infant 3 dyads, but particularly high in mother–daughter dyads (Fig. 1).

The complete model of generic contact was significantly different from the null model (LRT generic contact $\chi^2_3 = 14.5$, $P = 0.01295$). Variation in contact time was explained by maternal physical condition and by the interaction between maternal physical condition and offspring sex (Table 4). Specifically, contact time decreased with increased maternal physical condition (Fig. 2a), and whereas mother–son dyads spent less time in contact when mothers had high physical condition, mother–daughter dyads spent more time in contact when the maternal physical condition increased (Fig. 2b).

The complete model for proximity was significantly different from the null model (LRT proximity $\chi^2_3 = 13.2$, $P = 0.021$). Variation in proximity time was explained by the interaction between maternal physical condition and offspring sex (Table 4). Whereas mother–son dyads spent more time in proximity when maternal physical condition increased, mother–daughter dyads spent less time in proximity when mothers were in better condition (Fig. 3).

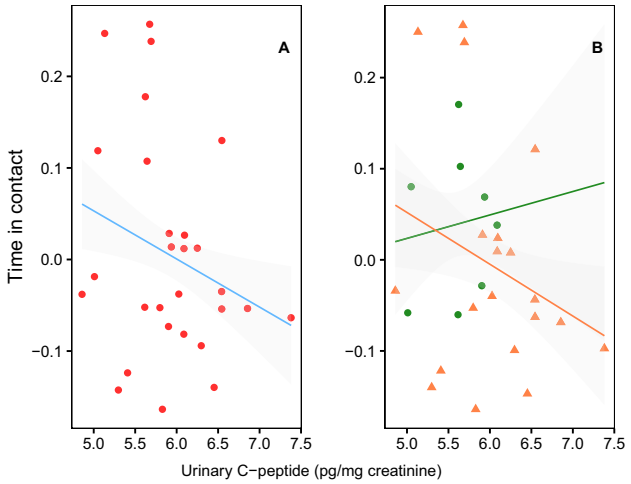

Fig. 2 Factors affecting time in contact in mantled howler monkey (*Alouatta palliata*) mother–offspring dyads: **a** maternal physical condition; **b** the interaction between maternal physical condition and offspring sex. Time in generic contact is depicted as the residuals of the complete model excluding the variable of interest. Areas shaded in gray represent 95% confidence intervals. Green circles are daughters, and orange triangles are sons

Discussion

In this study, we examined the influence of the physical condition of mothers and offspring sex on maternal care exhibited by female mantled howler monkeys. Dorso-ventral contact and vigilance were not explained by maternal condition and offspring sex. Mothers in better physical condition spent less time in generic contact with their sons but more with their daughters. For proximity, the relationship was the opposite: mothers in better physical condition spent more time in proximity with their sons but less time with their daughters. Independently of maternal physical condition, mothers tended to spend more time in ventro-ventral contact with their daughters than with their sons, especially at the

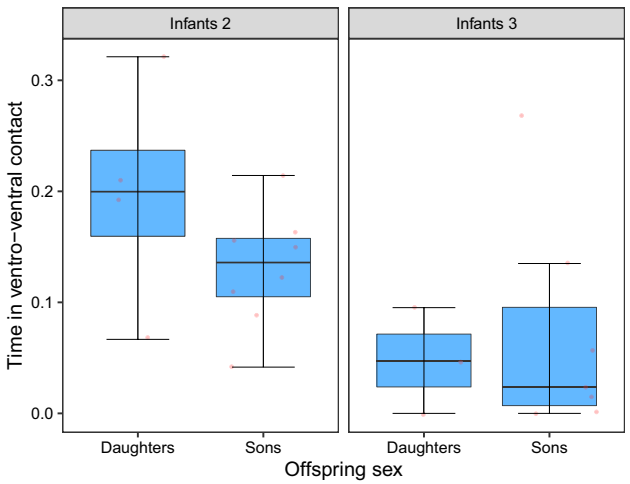

Fig. 1 Variation in time spent in ventro-ventral contact as a function of offspring sex and infant age in mantled howler monkeys (*Alouatta palliata*). Time in ventro-ventral contact is depicted as the residuals of the complete model excluding the variable of interest. Center lines show the medians, box limits indicate the 25th and 75th percentiles, and whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles

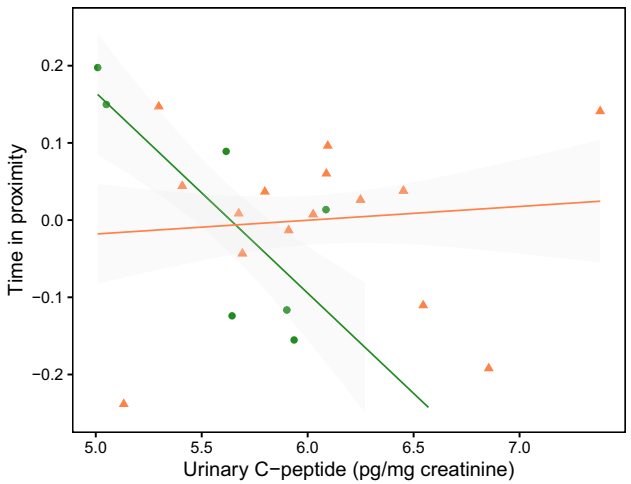

Fig. 3 Variation in maternal proximity time as a function of maternal physical condition and offspring sex in mantled howler monkeys (*Alouatta palliata*). Green circles are daughters, and orange triangles are sons. Time in proximity is depicted as the residuals of the complete model excluding the interaction between maternal physical condition and offspring sex. Areas shaded in gray represent 95% confidence intervals

infant 2 age. Thus, our prediction was not met, suggesting that the Trivers–Willard hypothesis (TWH) does not explain variation in maternal care in mantled howler monkeys for these behaviors.

Time in ventro-ventral contact decreased with infant age for both sexes. Given that the interactions between maternal physical condition and offspring sex did not affect this behavior, we assume that its variation is not explained by the TWH. However, this was the only behavior with significant variation between age categories. The decrease in ventro-ventral contact time could be the outcome of a decline in lactation as offspring grows (Balcells and Veà 2009; Dias et al. 2018).

The postpartum investment prediction of the TWH has been extensively tested, both empirically (Bereczkei and Dunbar 1997; Cameron and Linklater 2000; Cronk 1989; Fujita et al. 2012; Labov et al. 1986; Wright et al. 1988) and theoretically (Borgstede 2019; Veller et al. 2016). Still, results have not been conclusive (Brown 2001; Cronk 2007; Hewison and Gaillard 1999; Lonsdorf 2017; Keller et al. 2001), especially when within- and between-species comparisons are performed (McClure 1981; Sikes 1995). The TWH states that investment should be biased toward the offspring that yields higher fitness returns to parents per unit invested. However, it has been argued that it is implausible for natural selection to have shaped investment patterns on current offspring based on information only available in the future (Cronk 2007). Also, "maternal condition" was not explicitly defined by Trivers and Willard (1973), which has led to the use of a myriad of measures of female condition (Clancey and Byers 2014; Dias et al. 2018). For properly testing the TWH, it is important to use actual measures of female physical condition, such as C-peptide concentrations that proxy energetic balance (Emery Thompson 2017), rather than indirect measures, such as female dominance rank (Silk and Brown 2004). Finally, as researchers have failed to model the heritability component of the TWH in a population genetics framework, the evolutionary dynamics and genetic basis of this model remain elusive (Veller et al. 2016).

Given previous calculations of higher variance in reproductive success among males than females in mantled howler monkeys (Clarke and Glander 1984), according to the TWH, we expected that mothers in better condition would care more for their sons than for their daughters. However, lifetime reproductive success estimates for the Costa Rican population studied by Clarke and Glander (1984) were mainly based on data from a group that comprised a mean of 3.3 males (range = 2–4) and 7.7 females (5–10), whereas our study groups were larger, with a mean of 5.5 males (2–9) and 8.1 females (2–16). Additionally, in their calculations, Clarke and Glander (1984) assumed that alpha males had exclusive access to females in their groups, but at Los Tuxtlas and elsewhere no such exclusivity has been observed (Dias et al. 2010; Wang and Milton 2003). This variation in demographic and social attributes may affect individual reproductive success (Altmann and Altmann 1991; Perret 1990, 1996). Therefore, it is possible that in our population the fitness returns accrued through sons are not higher than those obtained through daughters.

Contact between mothers and their offspring is associated with the benefits of transportation and protection from predators, conspecifics, and adverse weather (Altmann 1980; Koban et al. 2003; Silk 1980). Additionally, contact time is positively associated with suckling and carrying (Johnson and Southwick 1987); hence rejection of contact is used by the mother to promote infant independence (Barrett et al. 1995). However, it is also associated with many costs. In rhesus macaques (*Macaca mulatta*), while in contact with their offspring, mothers face decreased likelihood of engaging in other behaviors, such as feeding or grooming (Altmann 1980; Rosenblum and Sunderland 1982). Proximity, in contrast, also benefits offspring but involves a lower cost to mothers than contact, as mothers are still able to perform other behaviors. Additionally, in both catarrhine (Altmann 1980; Förster and Cords 2004) and platyrrhine monkeys (Chism 1986), infants play a major role in maintaining proximity, and thus the costs of this behavior are low for mothers.

Although mantled howler monkey males have faster growth rates than females during the first four years of life (Raguet-Schofield and Pavé 2015), differences between sexes in growth are more pronounced after the first year, when most maternal care occurs (Connour and Glander 2020; Froehlich et al. 1981). Thus, the physical condition that offspring accrue during the first year of life may not be critical to determine adult physical condition. This would not support the second argument of the TWH, which states that differences in physical condition among offspring at the end of the parental investment period will endure into adulthood. Additionally, mantled howler monkey sons become independent from their mothers at an earlier age than daughters (Clarke and Glander 1984). Independence in this species is associated with increasing distance from the mother (e.g., Arroyo-Rodríguez et al. 2007; Baldwin and Baldwin 1973), as observed in other primates (Bentley-Condit 2003; Nguyen et al. 2012). Therefore, variation in contact time and proximity could result from divergent behavior between sexes rather than from bias in maternal care (Clutton-Brock 1991; Ono and Boness 1996).

Alternatively, increased contact at high maternal physical condition could reflect adaptive bias in investment toward daughters. The local resource competition hypothesis posits that mothers will invest more in the sex that disperses to avoid competition for limited resources (Clark 1978). Accordingly, there is evidence that within-group genetic relatedness in mantled howler monkeys is

higher among males than among females, suggesting that the former are less likely to emigrate than the latter (Milton et al. 2016; Niddifer and Cortés-Ortiz 2015), resulting in stronger competition for resources between mothers and their sons than with their daughters. Additionally, in other howler monkey species interbirth intervals are longer following the birth of daughters, which is suggestive of higher maternal investment (Dias et al. 2016), and females produce more daughters than sons when conditions are favorable (high rainfall, low population density, low glucocorticoid hormone concentrations: Dias et al. 2020; Rangel-Negrín et al. 2018). Even if the rearing of males is costlier (e.g., sons begin consuming solid food later than daughters and have faster growth rates: Raguet-Schofield and Pavé 2015), sex-specific patterns of infant growth are not necessarily produced by greater maternal investment (Clutton-Brock 1991; Geary et al. 2003), but may be due to differential maternal investment strategies (Hinde 2009). Moreover, females in good physical condition could achieve higher fitness benefits through their daughters because sons may mate with all available females in their group, including reproducing with low-quality females that will not be able to rear successful offspring (Leimar 1996). Still, given that differential maternal care according to infant sex was observed in two of the five behaviors that were studied, it remains for future research to determine whether the observed patterns correspond to adaptive sex allocation.

In conclusion, we found evidence that in mantled howler monkeys, some maternal care behaviors are biased in favor of daughters. This evidence converges with contentions that howler monkeys show adaptive sex allocation strategies (Dias et al. 2020; Rangel-Negrín et al. 2018). In light of the current maternal investment models (Leimar 1996; Trivers and Willard 1973; Veller et al. 2016), this suggests that mothers may accrue higher fitness benefits through their daughters than through their sons.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10329-020-00883-6.

Acknowledgements We thank P. Cruz-Miros, Don J. Landa, and several volunteers for their help during fieldwork. We thank La Flor de Catemaco S.A. de C.V., Gen. J.A. González de la Fuente, and various landowners at Balzapote for granting access to their properties. Ing. J.L. Ponce Puente facilitated our work at La Flor de Catemaco, and A. Molina provided vital administrative support to our project. We thank F. Aureli, S. Torre, J.C. Bicca Marques (Associate Editor), and two anonymous reviewers for providing invaluable feedback to the work. This study was supported by Consejo Nacional de Ciencia y Tecnología (grants 866671/629266 and ciencia básica 254217). This study was noninvasive and complied with the guidelines of the Primate Society of Japan. Research protocols were approved by the Secretaria de Medio Ambiente y Recursos Naturales (permits SGPA/DGVS/0381/17) and adhered to the legal requirements of the Mexican law (NOM-059-SEMARNAT-2010). A. de la Torre would like to extend her sincere gratitude to Dario de la Torre and Mary Paz Herrera for support. P.A.D. Dias and A. Rangel-Negrín thank Mariana and Fernando for constant support and inspiration to study primate behavior.

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