Stable isotopes reveal the effects of maternal rank and infant age on weaning dynamics in wild chacma baboons

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ABSTRACT

Maternal strategies reflect the trade-off between offspring needs and maternal ability to invest, a concept described by the evolutionary theory of parent-offspring conflict. In mammals this conflict has often been investigated by studying weaning, the transition from maternal milk consumption to dietary independence. An investigation of individual variation in weaning can provide information on the adaptive significance of maternal strategies in relation to social and biological variables. We analysed nitrogen stable isotopes of hair samples collected from 22 mother-infant dyads in a wild population of chacma baboons (*Papio ursinus*), in conjunction with behavioural data on suckling, to explore the temporal dynamics of weaning, as well as the extent and determinants of individual variation in these dynamics. The weaning pattern suggested by isotope values and behavioural data were congruent. The difference between infant and mother stable nitrogen isotope values decreased faster with age in infants of low-ranking mothers, which suggests a faster progression towards weaning, perhaps due to subordinate females experiencing lower resource availability and so being less able to bear the costs of lactation over prolonged periods. Additionally, within-infant variation in stable nitrogen isotope values showed an increase with age (which was not detectable between infants), potentially highlighting the nutritional costs that weaning imposes on offspring. Our combination of isotope analysis and behavioural data from a wild population provides insight into the evolution of maternal strategies. In particular, it suggests that the quantity of care a mother can provide is affected by her rank, with subordinate females possibly not able to lactate for as long and perhaps benefiting from weaning earlier.

Key words: Breastfeeding; Maternal strategy; Nitrogen stable isotopes; Chacma baboons; Dominance rank

Mammalian reproduction involves obligatory maternal care to ensure an offspring’s immediate survival and growth, and potentially enhance their future reproductive success and longevity (König and Markl 1987). Yet maternal care entails costs that can impact a female's ability to reproduce in the future (Trivers 1972; 1974). The evolutionary trade-off between investing in current offspring and saving energy for future reproduction generates a conflict: while mothers are expected to adjust the amount of maternal care they provide, weighing up the benefits for current offspring against the costs for future progeny, offspring are expected to prioritize their own fitness and maximise the level of maternal care received (Trivers 1974).

In mammals, lactation is considered the most energetically demanding stage of maternal investment, after which mothers usually resume their cycle and reproduce again (Thompson et al. 2012). The process of offspring gradually reducing their reliance on maternal milk to gain nutritional independence is called weaning (Trivers 1974; Martin 1984). Decreased reliance on milk may reflect a progressive quantitative decline in milk consumption, or alternatively a stable milk dependency throughout development associated with a progressive increase in the consumption of solid food (whereby lactation ceases suddenly at the end of the weaning process) (Van Noordwijk, Kuzawa and Van Schaik 2013). Hence, weaning is a change in offspring dependence on their mother as a function of time (Martin 1984), and variation in this reflects variation in the mothers' and offspring's strategies adopted to overcome the parent-offspring conflict (Trivers 1972; Lee 1987; Maestripieri 2002). Weaning dynamics directly affect the maternal reproductive trade-off, with earlier weaning leading to shorter inter-birth intervals and higher reproductive rates in mothers, but poorer survival, growth and fitness in the weaned offspring (Trivers 1974; Thompson et al. 2012). Consequently, studying individual variation in the timing of weaning and assessing which ecological, maternal or infant characteristics lead to this variation, is informative for our understanding of demographic processes and the evolution of mother-offspring relationships.

Parental investment theory (Trivers 1972) predicts that individuals should care for their current offspring over a longer period of time when they are less likely to reproduce again in the future, or when they need to spread the costs of lactation so they can better afford them on a daily basis. The first case often applies to older females, with the "terminal investment hypothesis" (Williams, 1966) predicting increased care for the last offspring of their reproductive career. In the second case, the "targeted investment hypothesis" (Cameron et al. 2000) suggests that mothers in poor condition who are limited in the daily energy they can invest in care, may compensate by allocating a longer period of care to their offspring. For example, this would be expected within social groups in low-ranking mothers as they are less able to access high-quality resources and so are in worse physical condition (Silk et al. 1981; Arlet et al. 2014). Finally, maternal investment should also be directed towards the offspring sex that will benefit most from it (Trivers and Willard 1973; Reitsema et al. 2016; Bădescu et al. 2022). According to the "fragile male hypothesis", sons are physiologically more vulnerable to environmental stress and increased lactation effort may improve their survival chances (Lee and Moss 1986; Wells 2000). Mothers might also obtain inclusive fitness benefits from investing in sons, in species where males generally have the potential for greater reproductive success compared to females (Trivers and Willard 1973; Hewison and Gaillard 1999). Finally, in sexually dimorphic species, male offspring usually need to grow more than females to reach full adult size, and so often receive more maternal care (Trivers and Willard 1973; Hewison and Gaillard 1999; Fedigan et al. 2008; Gesquiere et al. 2018).

Most studies investigating weaning have been based on life-history data, such as length of lactational anoestrus, and on behavioural data looking at how suckling activity decreases with age, e.g., African elephants (*Loxodonta africana:* Lee and Moss 1986), pinnipeds, ungulates and primates (Lee, Majluf and Gordon 1991), hooded seal (*Cystophora cristata*: Lee 1996), chacma baboons (*Papio ursinus:* Johnson 2003), rhesus macaques (*Macaca mulatta:* Hinde et al. 2009a), African elephants (*Loxodonta africana africana:* Wisniewska et al. 2015), mice (*Mus musculus:* Franks, Champagne and Curley 2015), mountain gorillas (*Gorilla beringei beringei:* Eckardt et al. 2016), yellow and olive baboons (*Papio cynocephalus and P. anubis*: Gesquiere et al. 2018), and Eastern chimpanzees (*Pan troglodytes schweinfurthii:* Lonsdorf et al. 2019). Yet behavioural observations of suckling time and nipple contact may overestimate weaning age (Lonsdorf et al. 2019; Bădescu et al. 2017) because they are unable to distinguish “nutritional nursing” from “comfort nursing”, i.e., oral contact with the nipple without milk transfer (Martin 1984; Borries et al. 2014). It is also possible that comfort nursing increases with age, with nipple contact serving more as pacifier than as a source of nutrition (Martin 1984; Borries et al. 2014). Additionally, behavioural data may underestimate weaning age by only reporting diurnal suckling bouts and not accounting for nursing time during the night, when observations are not normally conducted (Reitsema 2012).

A more reliable approach to investigate variation in weaning time uses stable isotope signatures (Higgins et al. 1988; Cameron et al. 1998; Fuller et al. 2006; Newsome et al. 2006; Dalerum et al. 2007; Crowley 2012; Reitsema 2012; Reitsema et al. 2016; Bădescu et al. 2017). This method is based on the principle of “you are what you eat”, because the body’s isotope composition reflects its diet over the period of tissue synthesis (Fuller et al. 2006; Reitsema 2012; Oelze et al. 2016). Nitrogen stable isotopes reflect the nitrogen content of amino acids in dietary proteins (DeNiro and Epstein 1981; Bădescu et al. 2017). They are measured using the ratio of ^{15}N to ^{14}N ($\delta^{15}N$), which reflects trophic position (DeNiro and Epstein 1981; Gannes, O’Brien and del Rio 1997; Bearhop et al. 2004) because, when N is incorporated into tissues, ^{14}N is excreted faster than ^{15}N (Steele and Daniel 1978). $\delta^{15}N$ values are useful for describing the weaning process since females produce the milk consumed by suckling infants, who thus occupy a higher trophic level than their mothers, with higher δ^{15}N values (Fuller et al. 2006; Newsome et al. 2006). In fact, the δ^{15}N values in tissues of suckling primate infants are approximately 2-3‰ higher than those of their mothers (Reitsema 2012; Tsutaya and Yoneda 2015). This mother-infant difference is expected to gradually decrease during the weaning process until it disappears entirely as infants stop consuming milk and consume a diet similar to their mother's (Reitsema 2012; Fahy et al. 2014). Stable isotope signatures can be measured in any tissue, but it may be valuable to use hair samples in weaning studies. This is because hair samples provide information over a time period of weeks to months (the time period over which hair is synthesised; Dalerum and Angerbjörn 2005), their collection is minimally invasive, and they are easy to store and metabolically inert, preserving the information indefinitely (Crowley 2012; Oelze 2016).

In this study we used stable isotope analysis to investigate variation in weaning age in wild infant chacma baboons (*Papio ursinus*). Chacma baboons are sexually dimorphic primates that live in multimale-multifemale groups, characterised by female philopatry, stable matrilineal hierarchies and promiscuous mating (Fischer et al. 2019). Maternal care in baboons is costly, with lactating females having reduced feeding time and body mass (Barrett, Halliday and Henzi 2006). In chacma baboons there is evidence that females face reproductive trade-offs, for example over the timing of birth which can either maximise offspring survival (current reproduction) or minimize maternal interbirth intervals (future reproduction; Dezeure et al. 2021a). It appears this birth timing trade-off then influences mother-offspring conflict after birth since infants born at times sub-optimal for their own survival throw more tantrums (Dezeure et al. 2021a). Increased maternal investment in baboons has been linked to maternal characteristics and environmental conditions, such as lower maternal rank (Johnson 2006), reduced food availability, or increased competition in larger groups containing more females (Altmann and Alberts 2003).

The goals of our study were twofold. First, we aimed to characterize the temporal dynamics of weaning at the population level using stable isotope measures, and then compare these to the dynamics estimated from suckling observations to compare the estimates produced by these two methods, and provide an accurate population-level estimate of weaning age. Second, we aimed to test hypotheses about individual variation in weaning time. We predicted that weaning would occur later in male offspring (who are costlier to raise;

Hewison and Gaillard 1999) and in those with older mothers (terminal investment hypothesis; Williams, 1966) or lower-ranking mothers (targeted investment hypothesis; Cameron et al. 2000).

METHODS

Study site

We collected data in central Namibia, at the Tsaobis Baboon Project (22°23'S, 15°45'E), during three field seasons (May-December 2006, July-December 2018 and April-July 2019). The Tsaobis environment is very arid (mean annual rainfall ± SD = 192 ± 143mm) and seasonal, with most rain falling between January and April (Dezeure et al. 2021a), when temperature often exceeds 40°C. The landscape is characterised by two habitats: open rocky desert and riparian woodland patches. The open desert is dominated by small herbs and dwarf bushes, such as *Monechma cleomoides*, *Sesamum capense*, and *Commiphora virgata*. The riparian woodland is dominated by large trees such as *Faidherbia albida*, *Prosopis glandulosa* and *Salvadora persica*, growing along the ephemeral Swakop River (Cowlishaw and Davies 1997). The baboons' diet predominantly consists of flowers, leaves, berries and pods (Cowlishaw 1997). We studied two wild troops of chacma baboons, named L and J (troop sizes: total individuals, adult females, adult males. 2006: L = 32, 9, 7 and J = 57, 16, 9; 2018: L = 75, 19, 4 and J = 58, 16, 4; 2019: L = 85, 19, 5 and J = 62, 16, 5). Both troops have been studied since 2005 and are habituated to the presence of researchers at close proximity. All adults and most juveniles are individually identifiable using ear notches and naturally occurring markings (Huchard et al. 2010).

Field methods

Hair collection

We used hair samples collected from 22 mother-infant pairs on October 12th (troop J, *N*=9) and 26th (troop L, *N*=5) 2006, October 9th (troop L, *N*=2) and 16th (troop J, *N*=3) 2018 and May 21st (troop L, *N*=3) 2019. On these dates the troops were undergoing routine capture to collect morphological data and biological samples. Individual baboons were captured at dawn in separate metal cages (c. 2m x 1m x 0.5m) that were baited with corn cobs. Mothers were sedated in the cage at the trapping site by a registered wildlife veterinarian with 100 mg/ml of tiletamine–zolazepam (Zolatil™Virbac Australia Ltd, NSW, Australia) at a dose rate of 5 mg/kg administered intramuscularly using a pole syringe. Infants were not anaesthetised and were transported with sleeping mothers in covered cages to a shaded processing area. The anaesthesia lasts 30-45 minutes and so mothers were put on a saline drip to prevent dehydration. Young infants (<6 months old) were usually caught in the same cage as their mother and not anaesthetised but held quietly in a blanket by one of the capture team members within sight of their sleeping mother. After the short period when the mother was processed (~10-15 minutes), the infant was immediately placed back with their asleep mother in the release cage. Older infants (> 6 months old) are more independent and often trapped independently from the mother. They were anesthetized using the protocol described above and usually placed in release cages with infants or juveniles that were approximately the same size in order to minimize stress during the waking-up period. In 2006, the processing site was located ~10 minutes away by car from the trapping site and individuals were transported in their cage, usually when asleep, to and from the processing site. In 2018-19, individuals were processed in the field, right by the trapping site. In all cases, the release cages were kept in the shade, close to each other, and covered with blankets, and the mother's recovery monitored closely. Care was taken to avoid placing mother-infant pairs, or young juveniles next to unfamiliar adult males. The entire social group was released together at the trapping site once all individuals were fully awake. Hair samples were collected from the thigh (2006) and subscapular (2018, 2019) areas, using sharp scissors to cut as close to the skin as possible. In order to ensure the presence of enough hair in the anagen phase to measure recent dietary intake and minimise the influence of hair in the telogen phase, we collected bulk of hairs from each individual to allow us to use at least the minimum number of hairs suggested by Mekota et al. 2006.

Behavioural data

We also collected behavioural data on suckling events from the same mother-infant dyads. We conducted focal observations of infants which lasted 60 minutes in 2006 (mean focal hours per individual ± SD=11.8 ± 1.1, *N*=13 dyads) or 20 minutes in 2018 (12.6 ± 1.0, *N*=4 dyads) and 2019 (14.8 ± 1.7, *N*=3 dyads). During focals we recorded whether the infant was suckling, foraging for solid food or engaged in another activity (e.g. locomotion) at five minute intervals. We used these scan observations to calculate, on a monthly basis for each infant, the proportion of feeding scans (suckling and foraging for solid food) in which an infant was observed suckling.

Data on maternal age and rank and infant age

Infant ages were easily estimated when birth dates were directly observed. When only the conception date was observed, we added the mean gestation length (*N*=190 days, range: 181-200 days, SD=5, *N*=13 pregnancies where both conception and birth were observed) to obtain the birth date. When neither conception nor birth were observed, the birth date was estimated using infant coloration, or, when this was uninformative, the reproductive state of mother in the preceding field season (see Dezeure et al., 2021b for further details on the estimation of birth dates in this population). Among the 22 infants considered in this study, the uncertainty over birth date ranged from 0 to 60 days (median = 10 days). Infants for which the birth date uncertainty was >60 days were not included in this study.

Maternal ages were estimated through dental eruption and molar wear patterns recorded in this population during trapping. Briefly, age up to the eruption of the last molar was estimated using Kahumbu and Eley’s (1991) eruption schedule. Beyond this age, molar wear rates were estimated from individuals captured at multiple time points and extrapolated across all individuals. These estimates have been validated by comparison of known age differences versus those estimated by molar wear in 19 individuals trapped multiple times over 1-5 years (but not included in the initial estimation of molar wear rates; see Huchard et al. 2009 for further details). Adult dominance ranks in each troop were established from *ad libitum* or focal observations of agonistic and approach-avoidance interactions. These were used to calculate a dominance hierarchy in Matman 1.1.4 (De Vries 1998). Landau’s linearity index showed that theses hierarchies were linear (2006, troop L: $h=0.95$, $X^2=63.63$, $P<10^{-4}$, troop J: $h=0.67$, $X^2=93.55$, $P<10^{-4}$; 2018, L: $h=0.25$, $X^2=26.11$, $P=0.089$, J: $h=0.38$, $X^2=37.83$, $P=0.016$; 2019, L: $h=0.31$, $X^2=33.26$, $P=0.027$). In order to control for differences in troop size between years and troops, relative rank was calculated as (N-absolute rank)/(N-1), with N the number of adult females in the group in a given year. This measure ranged from 0 to 1, with 0 for the lowest ranking female and 1 for the highest ranking.

Ethical note

This research adhered with the ASAB/ABS Guidelines for the Use of Animals in Research, the Namibian legal requirements and was carried out with the permission of the Ministry of Environment and Tourism, the Ministry of Land Reform, and the National Commission on Research, Science, and Technology. Our research procedures were evaluated and approved by the Ethics Committee of the University of Roehampton, the Ethics

Committee of the Zoological Society of London and the Ministry of Environment and Tourism, Namibia (MET Research / Collecting Permits 1039/2006, RPIV00392018/2019).

Hair sample preparation and analysis

Hair samples from the 22 mother-infant pairs were prepared for stable isotope analyses at the University of Roehampton. Hairs were washed in deionised water, scraped to remove any lipids, dirt and contaminants, and finally dried in the oven at 60°C for 30 minutes (Robertson et al. 2015; Sheppard et al. 2018). We avoided using chemical treatments to remove free lipids as these can distort carbon and nitrogen isotope ratios (Perkins et al. 2013, Elliot et al. 2014). Each hair was partitioned into 1cm sections, with each section representing approximately one month's growth in chronological order (the section nearest the root being the most recent; hair growth rates based on primate hair growth rates reported by Oelze 2016). Our analysis required the comparison of isotope values obtained from mother-infant pairs from the same time period. In each pair we obtained 3 to 5 segments (mean=4.09, *N*=22) from each individual. Each segment was homogenised using a scalpel and 0.7mg±0.1mg of this sample was weighed out and sealed into tin capsules (Elemental Microanalysis).

Previous isotopic analyses of weaning have used both δ^{15}N and δ^{13}C (^{12}C/^{13}C) isotope ratios (Reitsema 2012; Beaumont et al. 2015). However, δ^{13}C is more sensitive to habitat changes, gives less information about trophic discrimination and decreases to maternal levels more quickly than δ^{15}N (Fuller et al. 2006; Reitsema 2012). In our study, the difference between infant-mother δ^{13}C values decreased rapidly with infant age and so was not informative about variation in weaning age (supplementary materials: Figure S1). This is likely to be because, as suggested by Fuller et al. (2006), the drop of infants δ^{13}C values indicates the introduction of solid food in the diet rather than the duration of breast milk consumption, and infants start consuming solid food while still relying on breast milk.

Samples were run on a Europa Scientific 2020 isotope ratio mass spectrometer linked to a Thermoquest EA1110 elemental analyser running in continuous flow mode at Elemtex Ltd (Gunnislake, UK). Isotope ratios were expressed as δ values, using parts per million (‰) (O'Brien and Wooler 2007; Crowley 2012), according to the equation:

$$\delta X = (\mathrm{Rsample}/\mathrm{Rstandard}) - 1 \times 1000$$

where X represents ^{15}N and R the ratio of heavy to light isotopes (^{15}N/^{14}N). International reference materials (IAEA, Vienna) were also analysed within each run for calibration, with δ^{15}N samples values scaled to AIR. In order to assess the quality of these isotope data we checked that the C:N ratios ranged between 3.00 and 3.80 (O'Connell et al. 1999). The mean C:N for our hair samples was 3.43 (SD = 0.14, range = 3.11-3.79).

Statistical Analysis

1. Population-level weaning dynamics

We analysed population-level weaning trends using δ^{15}N stable isotope values following Reitsema et al.'s (2016) method to detect breastfeeding by comparing maternal and infant stable isotope values from the same time period. To do this we compared each infant's monthly δ^{15}N value with their mother's mean δ^{15}N value (Δ^{15}N) to control for variation in isotope values caused by changes in maternal diet (Reitsma et al. 2016). This comparison process involved four steps: (1) In each mother-infant pair, we calculated the mean δ^{15}N value for each mother across all hair sections. This mean value was calculated across all monthly infant values, to control for short-term fluctuations in the mother's diet during this period and provide a value representing baseline for each female. (2) We then subtracted this mean value from each infant's monthly δ^{15}N value to produce monthly Δ^{15}N values. (3) We then calculated the mean Δ^{15}N values across infants for each monthly infant age. Each monthly value was based on 3-12 infants (median 7, N= 12 months), with each infant contributing to 3-5 months (median = 4). We excluded values from ≤5 months and ≥18 months of age, as N = 1 for these age ranges. (4) Finally, we plotted the mean Δ^{15}N values against infant age to characterise the weaning process in this population.

In addition, we compared the trend described by Δ^{15}N values with the trend described by our suckling observations. Here, we calculated the mean proportion of feeding scans (suckling and foraging for solid food) in which an infant was observed suckling for each monthly infant age. We then compared the distribution of these proportions across monthly infant ages with the distribution of our Δ^{15}N values using Kolmogorov-Smirnov tests.

Finally, we estimated weaning ages for each individual by comparing the resulting Δ^{15}N values with the range of variation of δ^{15}N for mothers in this population, calculated here as the standard deviation of the mean maternal δ^{15}N (0.805). An infant was considered weaned when its Δ^{15}N values were equal or below to the standard deviation (for examples of these estimations, see supplementary materials: Figure S2).

2. Individual variation in weaning

We tested our predictions about the influence of offspring sex, maternal rank and maternal age on weaning by running two complementary models with normal error structure: (1) a linear model (LM) with weaning age estimates as response variable, and (2) a linear mixed model (LMM) with Δ^{15}N values as response variable. The first model provided a direct analysis of weaning age, but each infant only contributed a single point, and the sample was limited only to those infants for which we were able to estimate a weaning age (N= 12). The second model assessed progression towards weaning by measuring changes in Δ^{15}N values in relation to infant age. In this model, each infant contributed multiple points and the sample included all infants (N= 90 measures from 22 infants). Offspring sex, maternal rank and maternal age were included as fixed effects in both models. We included infant ID as random intercepts in our model of Δ^{15}N values to control for repeated measures (Mundry and Oelze 2016), while year and troop were included as fixed effects in both models to control for between troop and year effects. We also considered a possible quadratic effect of maternal age; females' IBIs are longer for both youngest and oldest individuals (Gesquiere et al. 2018), thus a similar effect could be found in terms of the length of the lactation period. To do so, we compared the models' performance using AICc values in the MuMIn package (Barton 2020). Models including a quadratic age effect always performed worse (higher AICc values - supplementary materials: Table S1) than those including a simple age effect, so we did not present these models here.

As infants get older and closer to being fully weaned, they reduce their reliance on maternal milk, and so their Δ^{15}N values are expected to decrease (Bădescu et al. 2017); thus we also included infant age in our model of Δ^{15}N values to control for this. However, in our dataset, age varied both within and between individuals as each infant was associated with more than one age value (corresponding to the different 1cm-sections of hair). This raised the possibility that the effect of age could be different within and between individuals. We therefore followed Van de Pol and Wright's (2009) within-subject centring technique. Here, the between-individual effect was estimated by fitting each infant's mean age across all of its Δ^{15}N values, whilst the within-individual effect was estimated by fitting the difference from each infant's mean age for each $\Delta^{15}N$ value.

In our model of $\Delta^{15}N$ values we fitted an interaction between each of the age variables, (i) between-infant age and (ii) within-infant age effect, and offspring sex, maternal rank and age to test our prediction that an infant's weaning is influenced by its own and its mother's characteristics. When these did not have a significant effect, we dropped them from our final model to allow us to test the significance of the main effects in these nonsignificant interactions (Engqvist 2005).

Since our first model predicting weaning age was fitted using a small data set ($N = 13$), there was potential for this to be overparametrized. Therefore, we ran three additional univariate models using maternal rank, maternal age and offspring sex separately to predict plotting weaning age estimates, with year and troop as random effects. To check for multicollinearity, we calculated the variance inflation factor (VIF) for each predictor in each full model using the 'vif' function in the 'car' package (Fox and Weisberg 2018). All VIFs were < 3 across all our final models. We checked that each model met the assumptions that the residuals were normally distributed and had homogeneous variance and that explanatory variables were independent.

We conducted all of our analyses using the program R version 3.6.1 (R Core Team, 2019), fitting the LM using the "stats" package (R Core Team, 2019), fitting the LMMs using the "lme4" package (Bates et al., 2015) and calculating models' r^2 values using the "MuMIn" package (Barton 2020).

RESULTS

1. Population-weaning dynamics

The mean values of $\Delta^{15}N$ in relation to infant age are shown in Figure 1a. Mean values of $\Delta^{15}N$ stayed relatively low from month 8, generally decreasing to month 17 describing the gradual process of weaning. Comparing the behavioural and isotopic measures of weaning, the proportion of feeding scans that infants spent suckling was relatively low between months 11 and 13 (mean ± SD: 0.0434 ± 0.0256, Figure 1b), but, in months 14 to 16 when $\Delta^{15}N$ values were at their lowest (Figure 1a), the mean time spent suckling was relatively high (mean ± SD: 0.227 ± 0.0531) before decreasing again at month 17. Supporting these observations, the $\Delta^{15}N$ distribution was significantly different from the distribution of the proportion of suckling time (Kolmogorov-Smirnov test: D=1.00, $P<10^{-4}$).

Estimation of weaning ages

It was only possible to estimate the weaning age for 13 out of 22 infants. For the remaining 9 infants, our hair samples did not capture the time period where Δ^{15}N values fell below the level of the between-mother variation (SD: δ^{15}N=0.805). For the 13 infants where this time period was captured, the mean (± SD) weaning age was estimated at 12.3 (± 2.5) months.

In the 9 infants where it was not possible to estimate a weaning age, 7 had Δ^{15}N values that remained greater than the level of between-mother variation, suggesting they had not weaned when we collected their hair samples. The remaining 2 were old enough to have started feeding away from the mother and closer to the father (Huchard et al. 2013), thus we suggest they had weaned before the time period our hair samples captured (supplementary materials, Figure S3). To confirm these assumptions, we performed Mann-Whitney tests, comparing the age distribution (i.e. the ages from each hair sample) of these two groups with the age distribution for the group for which we were able to estimate a weaning age. The infants who we suspected were not yet weaned were younger (mean age ± SD: 8.29 ± 2.14 months) than the infants for whom we estimated a weaning age (mean age ± SD: 12.57 ± 2.07 months; W=12.5, P=0.0088, N=19; Fig. S3). The infants who we suspected had weaned before our sampling period appeared older (mean age ± SD: 16 ± 2.73 months) than the infants for whom we estimated a weaning age, although the statistical comparison only approached significance (W=63, P=0.051, N=18; Fig. S3). Additionally, we confirmed that infants who we suspected were not weaned were younger than the infants who we suspected were already weaned (W=88, P=0.00051, N=19; Fig. S3).

2. Determinants of individual variation in weaning

Our weaning age estimates varied between troop and year, but were not predicted by maternal age, rank or offspring sex (Table 1). This is in line with the results of the additional univariate analyses that we ran in order to check that the results of our main model were not produced by overparameterisation (supplementary materials, Table S2). Our analysis of Δ^{15}N values indicated an interactive effect of rank and between-infant age (Table 1), such that Δ^{15}N values decreased with age for infants of lower ranking mothers whereas they were relatively stable for infants of higher-ranking mothers (Figure 2). Additionally, the Δ^{15}N values increased with within-infant age effect (Figure 3) potentially indicating an increase in nutritional stress, which we explore further below. Neither maternal age, offspring sex, troop nor year predicted Δ^{15}N values (Table 1). In order to account for the fact that the within-infant age effect may vary with age, we also ran a model including random within-individual age effect slopes for each individual, which confirms our results (supplementary materials, Table S3). The results for the non-significant interactions in the Δ^{15}N model are reported in the supplementary materials (Table S4).

DISCUSSION

We used stable isotope analysis to estimate weaning ages in wild chacma baboons and to test hypotheses of parental investment theory about individual variation in weaning. Our data estimate that weaning occurs in this population at a mean ± S.D. age of 12.3 ± 2.5 months. Contrary to our expectation, our findings suggest that the offspring of lower-ranking mothers progress towards weaning more quickly than those of higher-ranking mothers (between-infant effect). We also found that, as an infant gets older, it exhibits higher Δ^{15}N values, possibly an indication of nutritional stress (within-infant effect; Deschner et al. 2012; Bădescu et al. 2017). We did not find any support for the prediction that the offspring of older mothers wean later. Our study indicates the importance of a parent's social environment in the weaning process, and how this may impose nutritional costs on offspring. It also highlights the use of stable isotopes in measuring variation in this weaning process, especially in wild populations.

Individual variation in weaning

Our results highlight how social environment plays an important role in weaning, with the offspring of lower-ranking mothers moving towards nutritional independence faster than the offspring of high-ranking mothers (rather than more slowly as we predicted). This pattern was not observed in our analysis of weaning age, possibly because the weaning age model had much lower statistical power (both a smaller sample of infants and fewer data points per infant). Dominance enhances female reproductive success, with advantages such as priority access to food and offspring survival (Smuts and Nicolson 1989: Johnson 2003; Arlet et al. 2014), but the evidence for how rank affects weaning differs between studies (e.g. Gomendio 1990; Eckardt et al. 2016; Gesquiere et al. 2018; Lonsdorf et al. 2019), which presumably reflects the influence of other variables. In particular, ecological conditions can influence female weaning decisions (Lee et al. 1991; Altmann and Alberts 2003; Gesquiere et al. 2018), impacting maternal physiological state and hence affecting maternal strategy. Lee, Majluf and Gordon's (1991) comparative study across pinnipeds, ungulates and primates showed that, when food availability is more restricted, female mammals with relatively low nutritional status adopt an early weaning strategy, limiting how long they need to lactate. In contrast, late weaning is associated with a more abundant food availability, allowing females to sustain the costs of investment over a longer time period (Lee et al. 1991). Our study population lives in relatively harsh semi-desert environment (Cowlishaw and Davies 1997) and it appears that, in these conditions, lower-ranking mothers were less able to cope with the costs of lactation, and thus progressed towards weaning their infants earlier than more dominant females. Low-ranking mothers could alternatively, or in addition, have reduced the amount of maternal investment per unit of time they provide (Smuts and Nicolson 1989; Rachlow and Bowyer 1994). A lower amount of maternal care directly affects the maternal reproductive trade-off, and a faster weaning process has often been associated with shorter inter-birth intervals (IBIs) and lower offspring survival (Trivers 1974; Thompson et al. 2012). However, in this population lower-ranking females have longer IBIs than more dominant females (Dezeure et al. 2021a), despite the faster progression towards weaning we observed here. This shows the added benefit of isotopic data, suggesting that inferences on weaning age based on the length of lactational anoestrus (e.g. Johnson 2003; Gesquiere et al. 2018) may be misleading, because subordinate females may not be able to finance prolonged lactations and may subsequently need a longer time to replenish their energetic stores. Moreover, offspring survival, which is more important than IBI in influencing maternal lifetime reproductive success (Weibel et al. 2020), is not influenced by maternal rank in the Tsaobis population (Dezeure et al. 2021a), which suggests that rank influences maternal weaning strategy, but not necessarily female reproductive success. Another possible explanation is that low-ranking females accelerated weaning as a counterstrategy to infanticide from males (Colmenares and Gomendio 1988; Beehner and Bergman 2008), though it's not clearly established that subordinate females are more vulnerable than dominant ones in baboons. Male hierarchy instability may often increase infanticide risk (Baniel et al. 2018, Beehner et al. 2005), and our population was more stable in 2006 compared to 2018 and 2019, but our analysis did not show any year effect on weaning age. However, our sample only covers three different years and females may also engage in other strategies to protect their offspring from infanticide. For example, lactating female baboons can maintain close associations with males to gain protection for their infant (Palombit, Seyfarth and Cheney 1997; Weingrill 2000), and this behaviour may be particularly important for subordinate females with fewer social connections and in case of male takeovers (Beehner and Bergman 2008).

Our study also shows a within-infant increase in $\Delta^{15}N$ values with older age, which might be the result of greater nutritional stress rather than higher trophic position (Crowley 2012; Bădescu et al. 2017). Analysis of stable isotope signatures in multiple taxa has shown that $\delta^{15}N$ values are elevated under conditions of food deprivation (e.g. low protein diet, reduced food intake, poor environmental or physical conditions; McCue and Pollock 2008) and a recent study on hair samples showed for the first time isotopic evidence for nutritional stress in a free-ranging primate species (Oelze et al. 2020). When individuals experience a negative nitrogen balance (i.e. they are nutritionally stressed because they do not obtain sufficient nitrogen to meet metabolic requirements), they will start to catabolize their own tissues to synthesise the protein needed for survival, reincorporating liberated amino acids, enriched in ^{15}N compared to dietary ones, and thus causing an increase in $\delta^{15}N$ values (McCue and Pollock 2008; Deschner et al. 2012; Neuberger et al. 2013). Fuller et al. (2006) studied weaning by using stable isotope signatures from pairs of breastfeeding women and their nursing children and suggested that $\delta^{15}N$ values may be influenced not only by diet but also by nitrogen balance. This matches the established principle that the switch from maternal milk to solid food is nutritionally stressful because solid food is (i) per unit more difficult to digest and less nutritious compared to maternal milk, which contains approximately 16mg/ml protein (Buss 1968), (ii) it is higher in nitrogen content compared to maternal plant-based food (Reitsema 2012), and (iii) more difficult to find and exploit, since infants need to learn foraging skills and compete with the others for food resources (Rhine et al. 1985; Maestripieri 1995). In fact, the transition from exclusive reliance on milk to reliance on solid food may cause infants to be nutritionally stressed, although this has rarely been explored (Franks, Champagne and Curley 2015). The high $\delta^{15}N$ values caused by nutritional stress is indicated by a relatively low dietary nitrogen content (%N), whereas infants with the high $\delta^{15}N$ values caused by milk consumption should have higher %N (Bădescu et al. 2017). Urea and faeces' %N values have been found to reflect dietary %N values (Deschner et al. 2012; Bădescu et al. 2017). Petzke et al. (2005) examined the content of human hair and suggested that the amount of hair ^{15}N may similarly reflect the dietary nitrogen content, but the link between hair and dietary %N values is less clear. Establishing such a link would allow studies using stable isotopes analysis of hair samples to investigate the physiological implications of weaning on offspring.

While we expected sons to wean later than daughters, as recently found in chimpanzees (Bădescu et al. 2022), we did not find any evidence of sex-bias investment in this study. Such a negative result may reflect the limitations of our sample size to detect small effect sizes. Another possible explanation is that that male and female infant baboon energetic and nutritional needs are comparable since they have similar body sizes with sexual dimorphism appearing once juveniles are nutritionally independent.. Finally, empirical evidence for sex biases in maternal investment has been hard to gather in wild primate populations, often producing mixed and inconsistent results, and it remains difficult to find general patterns fitting with theoretical expectations (Lee 1987; Brown 2001; Hinde 2009b).

Lastly, maternal age did not predict offspring weaning, contrary to our expectation that older females would wean their offspring later. The lack of support for this prediction may be because it is generated by the “terminal investment hypothesis” that females invest more care in their final offspring (Williams, 1966), but most mothers in our data were still likely to reproduce in the future. Indeed, female baboons have a median lifespan of 18.5 years (Tung et al. 2016 - maximum observed lifespan of 26.7 years), while in our sample the median maternal age ± SD was of 14 ± 4.03 years, with only two females older than 18 years and so potentially near the end of their lives.

In conclusion, in order to improve our understanding of weaning and weaning-induced nutritional stress, future studies should determine the hair growth rate in baboons and possibly further investigate the correlation between nitrogen hair content and the dietary one.

Population-level weaning dynamics

Our isotope data estimates a mean ± SD weaning age in this population of 12.3 ± 2.5 months (*N*=13). This is in line with anecdotal reports from this population that infants older than 1-year old do not necessarily die following their mother’s death, as we would expect for infants still reliant on their mother’s milk for nutrition (E. Huchard, pers. obs.). This weaning age estimate coincides with the marked drop in suckling we observed in offspring aged 11 to 13 months (Figure 1b). However, these behavioural data suggest an abrupt end to milk consumption between 10 and 11 months old, whilst the nitrogen isotope data indicate a more gradual transition (Figure 1a). This gradual process was probably obscured in the behavioural data by a reduction in milk supply before weaning (meaning infants received less milk per unit time of suckling), up to the point where infants needed to forage autonomously to meet their nutritional requirements and mothers may have actively discouraged suckling producing the steep drop in suckling rates at 10 to 11 months. Moreover, data on tantrum behaviour in this population supports our assumption that mothers may be actively preventing their offspring from suckling in this period in order to complete the weaning process (Dezezeure et al. 2021a). In contrast, the increased time spent suckling after month 13 most likely reflects comfort nursing: as weaning infants experience food deprivation and hunger, they start to suckle again but without gaining milk. Indeed, it has been suggested that infants try to cope with the pressure of the nutritional stress that weaning causes by increasing nipple contact post-weaning, even if mothers have stopped producing milk (Hinde et al. 2009a; Bădescu et al. 2017; Bădescu et al. 2022).

Previous studies tend to analyse weaning by using either behavioural data, e.g., chacma baboons (Johnson 2003), house mice (*Mus musculus:* Franks, Champagne and Curley 2015), and Eastern chimpanzees (Lonsdorf et al. 2019), or isotopic data, e.g, northern fur seals and California sea lions (*Callorhinus ursinus* and *Zalophus californianus:* Newsome et al. 2006), Francois' leaf monkey (*Trachypithecus francoisi*: Reitsema 2012) and rhesus macaques (Reitsema et al. 2016), most of which were also performed on captive populations with an abundant food supply. Although some studies have attempted to correlate measures of time spent suckling with estimates of infant reliance on maternal milk (see the review by Cameron 1998) and with isotopic methods (*Eumetopias jubatus:* Higgins et al. 1988; *Equus caballus*: Cameron et al. 1999), the approach that we have adopted here builds on literature combining isotopic and behavioural data to study maternal strategies (e.g. Cameron et al. 1999; Bădescu et al. 2016; Bădescu et al. 2017; Reitsema et al. 2020; Bădescu et al. 2022), and indicates how these two types of data can be combined in wild populations to provide a more complete picture of the weaning process.

Our weaning age estimate of 12.3 ± 2.5 months in our chacma baboon population is in line with other baboon populations. Analyses of postpartum amenorrhea periods suggested that lactation lasted 10.4 ± 2.8 months in the yellow baboons of Amboseli, Kenya (*Papio cynocephalus:* Gesquiere et al. 2018), and 13.1 ± 0.6 months in the olive baboons of Gilgil, Kenya (*P. anubis:* Smuts and Nicolson 1989). Similarly, observations of nipple contact time in olive baboons at the Gombe Stream National Park, Tanzania, suggested weaning ages between 12-18 months (Nash 1978). Nevertheless, different environmental conditions can be a source of variation in the weaning period (Lycett et al. 1998). For example, in chacma baboons who lived in the Drakensberg Mountains, South Africa, where environmental conditions are relatively harsh, offspring spent more time on the nipple and started weaning around 7 months later compared to several East African baboon populations in more benign environments (Lycett et al. 1998). The semi-desert environment at Tsaobis is also relatively harsh, but our weaning estimate is closer to those reported for East Africa than for South Africa. As we found that low-ranked mothers-offspring pairs progressed towards weaning earlier, this may suggest that the costs of harsh conditions disproportionately affect low-ranked females rather than all individuals in the population. However, it is important to take into account methodological variations when making such comparisons. In particular, we know from this study that nipple contact may continue for some months after the completion of weaning, so using suckling behaviour to determine weaning is likely to lead to substantial overestimates. In addition, comfort nursing may be more frequent in harsh environments where weanlings are more nutritionally stressed, and mothers in such environments may also resume cycling more slowly if it takes them more time to recover their condition. Altogether, these complexities make it hard to compare across studies when weaning estimates are based solely on behavioural or life-history measures.

CONCLUSION

We used stable isotope values to measure variation in weaning in chacma baboon infants and to test hypotheses from parental investment theory. Our results suggest that the offspring of lower-ranking mothers move towards nutritional independence faster than the offspring of higher-ranked mothers, potentially due to differences in subordinate and dominant females' ability to sustain the costs of lactation. Our results also demonstrate the use of isotopic measures in reliably inferring weaning dynamics and age and how they can be used in parallel with behavioural data on suckling frequency from the same individuals. The weaning process is a key period for mammalian offspring development and our study highlights how, in highly social species such as baboons, this process can be influenced by a mother's social position.

ACKNOWLEDGMENTS

We thank the Tsaobis Baboon Project researchers, field assistants and volunteers from 2006, 2018 and 2019 for collecting behavioural data and hair samples, and we thank Iulia Bădescu and two other anonymous reviewers for their helpful comments and feedback on this paper. This research was carried out with the permission of the Ministry of Environment and Tourism, the Ministry of Land Reform, and the National Commission on Research, Science, and Technology. We further thank the Tsaobis beneficiaries for permission to work at Tsaobis, the Gobabeb Research and Training Centre for affiliation, and the Snyman and Wittreich families for permission to work on their land. The collection of the data we present in this study was organised within the Tsaobis Baboon Project, using the funding obtained by a grant from the Agence Nationale de la Recherche 616 (ANR ERS-17-CE02-0008, 2018-2021) awarded to Elise Huchard.

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Word count: 10216

TABLE

Table 1

Results of our models predicting estimated weaning age (*N*=13 infants) and Δ^{15}N values (*N*=90 samples from 22 infants for each measure).

Response variable	*Explanatory variable*	*Estimate*	*standard error*	*degree of freedom*	*T value*	*P-value*
WEANING AGE	Intercept	8.102	1.989			
R^2: 0.918	Maternal age	0.076	0.084	-	0.905	0.40033
	Rank	-0.322	1.048	-	-0.308	0.769
	Offspring sex: Male^a	0.972	1.040	-	0.935	0.386
	Troop: L^b	5.032	0.936	-	5.374	**0.002**
	Year: 2018^c	2.668	0.807	-	3.308	**0.016**
	Year: 2019^c	0.172	1.195	-	0.144	0.890
Δ^{15}N	Intercept	5.870	1.622			
R^2c: 0.859	Maternal age	0.036	0.043	12.917	0.835	0.419
	Rank	-6.997	3.056	12.959		
	Offspring sex: Male^a	-0.312	0.346	13.028	-0.902	0.384
	Troop: L^b	0.597	0.304	12.963	1.967	0.071
	Year: 2018^c	-0.088	0.494	12.783	-0.178	0.862
	Year: 2019^c	0.369	0.530	12.918	0.696	0.499
	Between-infant age effect	-0.440	0.143	12.972		
	Within-infant age effect	0.196	0.030	66.898	6.635	**<0.001**
	Rank x Between-infant age effect	0.618	0.254	12.922	2.435	**0.030**

Reference category = ^{a}Female; ^{b}J; ^{c}2006

R^2c is the conditional r^2 value (Nakagawa et al. 2017). Significant effects are marked in bold. The non-significant interactions were removed from the final models (Engqvist 2005) and are reported in the supplementary material (Table S3).

FIGURE LEGENDS

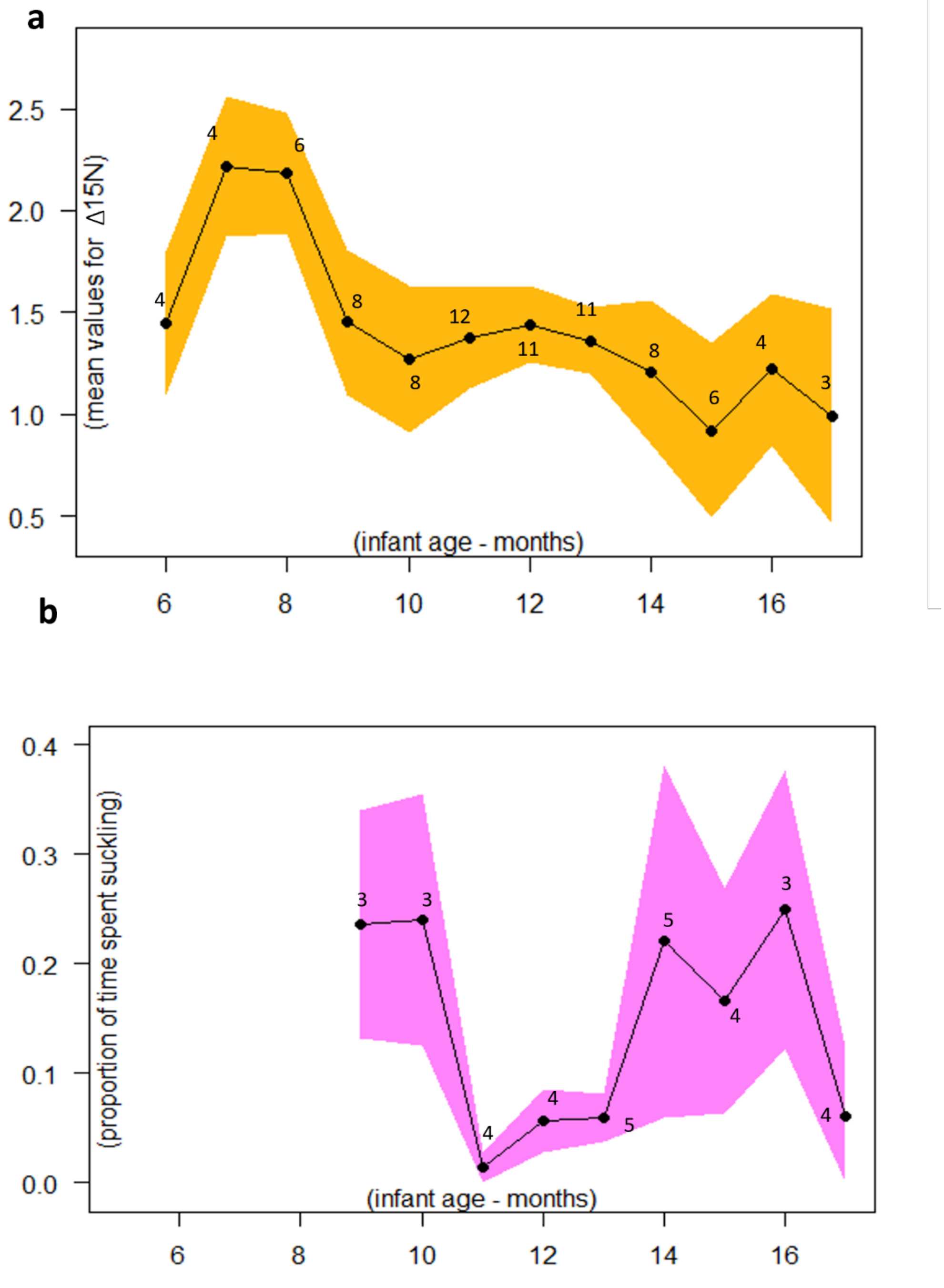
Fig. 1 Variation with age in (a) the mean difference between infant and mother $\delta^{15}N$ (Δ15N) and (b) the mean proportion of feeding scans in which infants were observed suckling. Standard errors are represented by a coloured area around the line. The numbers near each dot indicate the number of individuals contributing to each mean and standard error.

Fig. 2 The interactive effect of maternal rank and between-infant differences in age on $\Delta^{15}N$ values. The dots show infants with mothers whose rank is below (blue) or above (red) the median rank (0.667). The lines are the predictions of our model for the 25th (rank = 0.38, more subordinate mothers, red line) and 75th (rank = 0.80, more dominant mothers, blue line) percentile in our maternal rank data. In these predictions the within-age effect and maternal age are set to their mean, sex to female, troop to L and year to 2006. The shaded areas show the standard error around these predictions.

Fig. 3 The within-infant age effect on $\Delta^{15}N$ values. The line is the effect predicted by our model with all other continuous variables set to their mean, sex set to female, troop to L and year to 2006. The shaded area depicts the standard error around these predictions. In this figure zero represents each infant's mean sampling age, with positive values representing hair sections grown at ages older than the mean, and negative values representing hair sections grown at ages younger than the mean.

FIGURES

Fig. 1



763 **Fig. 2**

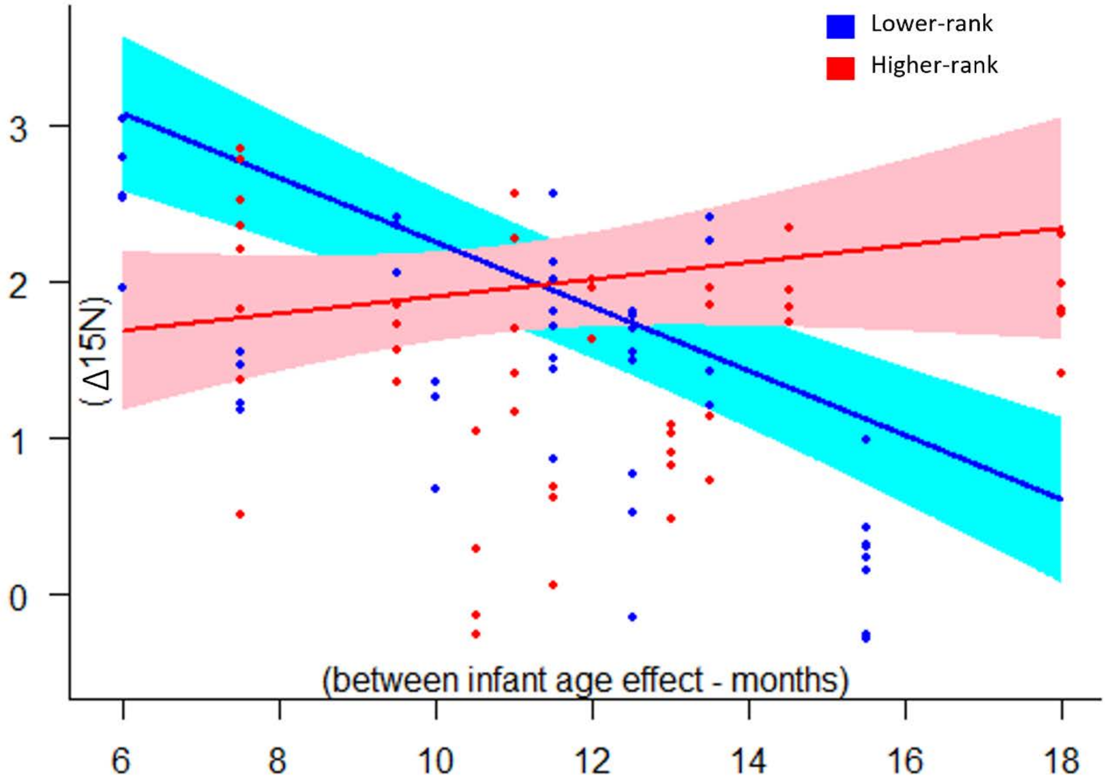
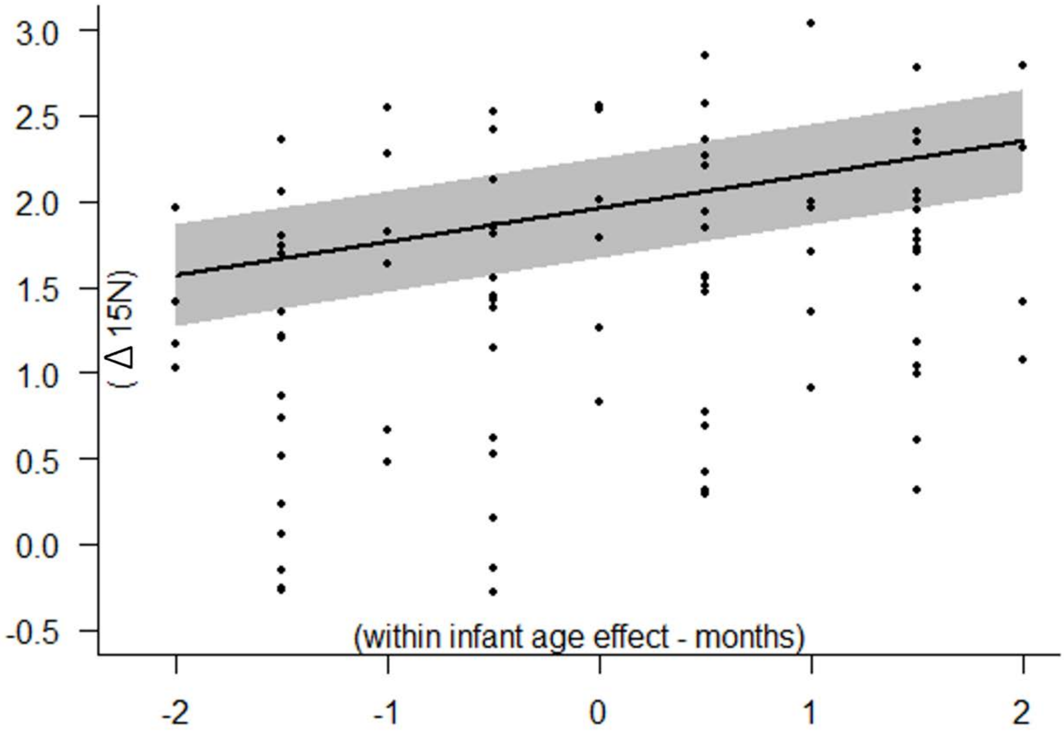

Lower-rank
Higher-rank
(Δ15N)
(between infant age effect - months)
3
2
1
0
6
8
10
12
14
16
18

Fig. 3


3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
(Δ 15N)
(within infant age effect - months)
-2
-1
0
1
2

SUPPLEMENTARY MATERIALS

Table S1. AICc values of the full models predicting weaning age and $\Delta^{15}N$ including either linear maternal age effect or quadratic (and a linear) maternal age as fixed effects.

Response variable	**AIC_c of the model including maternal age**	**AIC_c of the model including the quadratic effect of maternal age**
Weaning age	88.9	125.8
$\Delta^{15}N$	144.6	155.0

Table S2. Results of the models predicting estimated weaning ages (*N*=13 infants). Troop and year are random effects in all models.

Response variable	*Explanatory variable*	*Estimate*	*standard error*	*degree of freedom*	*T value*	*P-value*
WEANING AGE	Intercept	11.74	2.489			
	Maternal rank	0.094	0.065	-	1.448	0.190
WEANING AGE	Intercept	12.77	2.329			
	Maternal Rank	0.282	1.019	-	0.277	0.789
WEANING AGE	Intercept	13.03	2.485			
	Offspring sex: Male[a]	-0.219	1.393	-	-0.158	0.878

Reference category = [a]Female

Table S3. Results of the model including a random slope for the within-age effect predicting Δ^{15}N values (N=90 samples from 22 infants for each measure).

Response variable	*Explanatory variable*	*Estimate*	*standard error*	*degree of freedom*	*T value*	*P-value*
Δ^{15}N	Intercept	6.123	1.412			
R^2c: 0.897	Maternal age	0.038	0.037	12.737	1.019	0.327
	Rank	-7.672	2.642	12.903		
	Offspring sex: Male[a]	-0.418	0.3001	13.209	-1.392	0.187
	Troop: L[b]	0.601	0.262	12.868	2.291	**0.0395**
	Year: 2018[c]	-0.173	0.423	12.183	-0.410	0.689
	Year: 2019[c]	0.248	0.458	12.831	0.541	0.598
	Between-infant age effect	-0.443	0.124	12.934		
	Within-infant age effect	0.206	0.039	20.217	5.269	**<0.001**
	Rank x Between-infant age effect	0.655	0.219	12.792	2.990	**0.0106**

Reference category = [a]Female; [b]J; [c]2006

R^2c is the conditional r^2 value. Significant effects are marked in bold.

Table S4. Results of the non-significant interactions tested in our models predicting $\Delta^{15}N$ values (*N*=90 samples from 22 infants for each measure).

Response variable	*Explanatory variable*	*Estimate*	*standard error*	*degree of freedom*	*T value*	*P-value*
$\Delta^{15}N$	Rank x Within-infant age effect	-0.167	0.105	66.008	-1.592	0.116
	Maternal age x Between-infant age effect	-0.007	0.016	12.962	-0.442	0.666
	Maternal age x Within-infant age effect	0.001	0.008	66.009	0.135	0.893
	Offspring sex: Male[a] x Between-infant age effect	0.043	0.124	12.995	0.347	0.735
	Offspring sex: Male[a] x Within-infant age effect	-0.031	0.059	66.009	-0.514	0.609

Reference category = [a]Female

Figure S1. Variation with infant age in the mean difference between infant and mother $\delta^{13}C$ for each monthly pair of samples (hair sections from mother and offspring). Standard errors are represented by a coloured area around the main curve. The number near each dot indicates the number of mother-offspring pairs contributing to each mean and standard error.

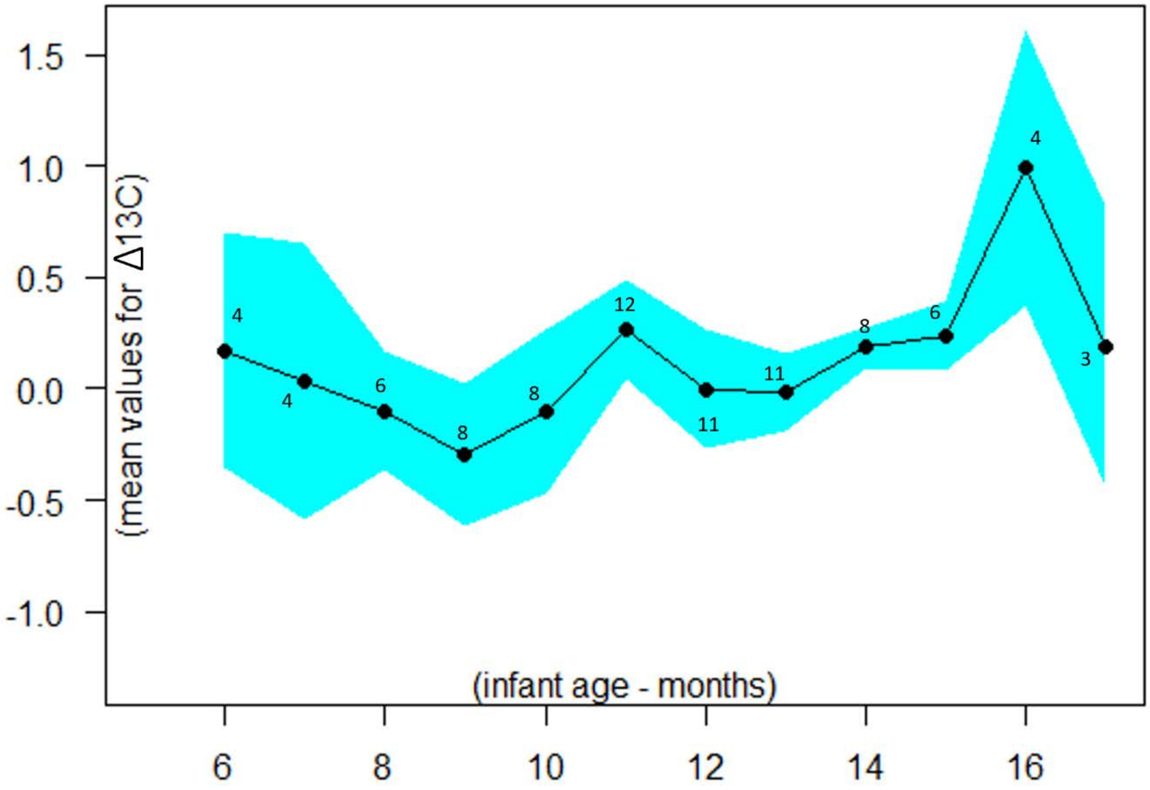


Figure S2. Example trajectories of $\Delta^{15}N$ values from three infants, compared to the between-mother standard deviation for $\delta^{15}N$ (0.805) (dotted horizontal lines in the figures) to estimate the infants' weaning ages. We were able to estimate weaning ages when $\Delta^{15}N$ values felt below the horizontal line. For example, the infant illustrated in panel a weaned at 12 months of age (green), while the other two infants were too young (panel b, in blue) or too old (panel c, in purple) for us to estimate the weaning age with these data. In fact, the main difference between panel b and c (besides the age of the two infants) is that the high $\Delta^{15}N$ values in panel b are probably due to consumption of milk whereas in panel c they are due to nutritional stress.

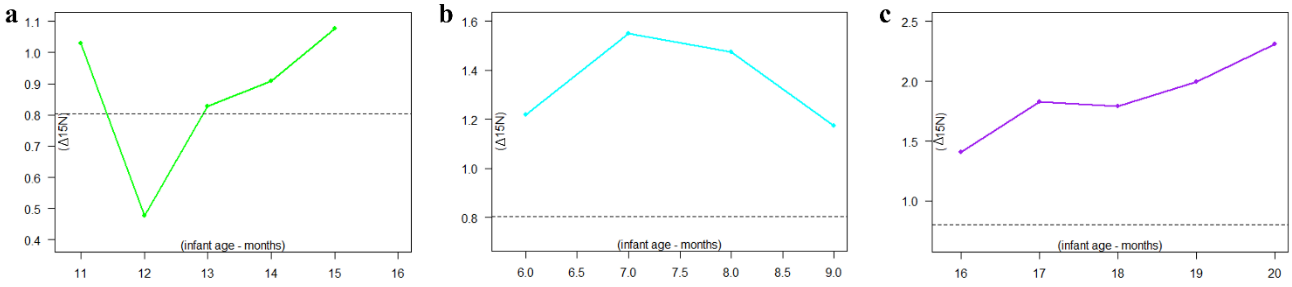


Figure S3. Age distribution (in months) of infants for whom we were able to estimate weaning age (green), and those who we suspected had already weaned before the time period our hair samples covered (blue) or had not yet weaned (purple). The mid-lines show the medians, the boxes the interquartile range, while the whiskers show 1.5 times the interquartile range. Each dot represents a monthly sample (i.e. one hair section) of each infant.