Title

The effects of pairing and reproduction on the sociality, physiology, and behavior of captive coppery titi monkeys (Plecturocebus cupreus)

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The effects of pairing and reproduction on the sociality, physiology, and behavior of captive coppery titi monkeys (*Plecturocebus cupreus*)

By

CHLOE LISABETH KARASKIEWICZ
DISSERTATION

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This dissertation is dedicated to my parents and grandparents, who gave me the world when they gave me my education.

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Απρ 11, 2023

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Abstract

For social animals, social relationships are massively important to an individual's ability to survive and thrive. Some relationships, like pair bonds, involve a high degree of attachment and interact with physiological processes like stress, sleep, and reproduction. The projects in this dissertation represent a cross-section of influential life events and processes throughout a pair-bonding primate's life and examine the impact of those events on the pair relationship and individual psychophysiology of captive coppery titi monkeys (*Plecturocebus cupreus*). In the first study, we observed nighttime activity as a behavioral proxy for sleep in newly formed pairs of titis to assess how they habituated to a new social and physical environment across their first week with a new mate. We also compared the frequency of nighttime and daytime affiliative behaviors between pair mates to assess the comparative frequency and role of such behaviors at different times of day in a diurnal species. In the second study, we examined the impact of hormonal contraceptive use on reproductive cycling and affiliation within pairs. The effects of physiological interventions are particularly important to understand in a laboratory setting where breeding is selectively managed, and relationships are studied in neuropsychological research. Finally, in the third study, we assessed the role of pregnancy and infant rearing on the relationship between co-parents and pair mates. Together, this work represents the first studies that quantify nighttime behavior, assess the impact of contraception, and consider the changing relationship between parenting partners in this diurnal, pair-bonding species.

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Abstract

Sleep is an important biological process that supports healthy physiological and psychological functioning. For social animals, social relationships also support survival and promote psychological well-being. Many social animals sleep with conspecifics and both the presence and relationship between sleeping partners can affect sleep. We observed nighttime activity and affiliation in $n = 12$ newly formed pairs of captive coppery titi monkeys (*Plecturocebus cupreus*) across their first week together to investigate how habituating to a new social and physical environment would affect nighttime behavior. Overall, titi monkeys were largely inactive during the dark period (18:00-06:00), with activity detected in only 10% of observations. Activity decreased across the pairs' first week together ($t_{(134)} = 1.99$, $p = 0.048$, $\beta = 0.16$). Activity was not sensitive to sex, age, or prior pairing experience. When individuals were active, they primarily engaged in vigilance behaviors (62% of active observations) and/or postural readjustments (25% of active observations). Affiliation was consistently high across our testing period, with pairs in affiliative contact in 73% of observations, and was sensitive to time of day, such that pairs engaged in significantly more affiliative behavior during the dark period, $X^2_1 = 68.19$, $p < 0.001$, $d = 2.22$, *95% CI*: [-2.66, -1.78]. Overall, activity was consistently low, and affiliation was consistently high during the dark period, which is consistent with other small-bodied, socially sleeping, diurnal primates. This work is the first to quantify nighttime behaviors in titi monkeys and assess them in the context of early pair bond formation.

Introduction

Sleep is a fundamental biological process, which supports physical (Mullington et al., 2010) and psychological health (Killgore, 2010), and encompasses more than a third of the average primate's life (Nunn & Samson, 2018). Despite this, sleep has been quantified in less than 10% of primate species (Campbell & Tobler, 1984; Sri Kantha & Suzuki, 2006). Several indices of physiological health are connected to sleep duration and quality including inflammation (Kuhn et al., 1969; Mullington et al., 2010; Toth et al., 1993), risk of heart attack (Sauvet et al., 2010), blood pressure (Ogawa et al., 2003; Zhong et al., 2005), and obesity (Cappuccio et al., 2008; Miller & Cappuccio, 2007). Psychologically, sleep supports a number of critical functions including sensory perception (*visual*: Kendall et al., 2006; *auditory*: Horne et al., 1983; Babkoff et al., 2005; *olfactory*: Killgore & McBride, 2006; Mcbride et al., 2006; *tactile and noiception*: Kundermann et al., 2004; Haack et al., 2009), attention (Dawson & Reid, 1997; Thomas et al., 2003), inhibitory control (Drummond et al., 2006; Killgore et al., 2006; Mckenna et al., 2007), learning (Drummond et al., 2000; Yoo et al., 2007), memory (Harrison & Horne, 2000; Yoo et al., 2007; Thomas et al., 2003; Mu et al., 2005), and affective processing (Kahn-Greene et al., 2006, 2007; Tempesta et al., 2010; Killgore et al., 2008). While impactful, these consequences do not exist in a vacuum, particularly for social animals that perceive, process, and respond to stimuli in the context of conspecifics and their relationships.

In group-living species, an individual's ability to survive and thrive can hinge upon social relationships (Silk, 2007). Social relationships shape how individuals see the world: from perceiving challenges (Schnall et al., 2008) to perceiving threats (Coan et al., 2006), relationships allow individuals to share the burdens of their goals and the resources to achieve them (Coan & Sbarra, 2015). Close social relationships can also support good physical health (Holt-Lunstad et al., 2008; Reblin & Uchino, 2008; Silk et al., 2010) and protect against health risks associated with loneliness (Cacioppo et al., 2010; Hawkley & Capitanio, 2015). As partner presence and components of the relationship can alter individuals' health and behavior, it should come as no surprise that who an animal sleeps with can affect their sleep quantity and quality. Good quality relationships support better sleep (Drews et al., 2017) and are associated with fewer symptoms of insomnia (Strawbridge et al., 2004; Prigerson et al., 1999; Troxel et al., 2009). Relationship satisfaction is also associated with higher sleep concordance (i.e., sleeping and waking with one's partner) (Gunn et al., 2015) and attachment insecurity is associated with poor sleep quality (Carmichael & Reis, 2005; Troxel et al., 2007).

Disruption of typical sleeping circumstances can also disrupt sleep. Macaques that were separated from their typical physical and social environment for a night of sleep observation displayed fragmented, highly disrupted sleep characterized by frequent wakefulness and shortened bouts of continuous sleep (Benca et al., 2000). Similarly, human couples separated during travel reported increased sleep problems while separated, compared to pre-separation and post-reunion (Diamond et al., 2008). This suggests that removal of important social sleeping partners and the familiar physical environment are sufficient for significant sleep disruption. Like much of the literature on coregulation in romantic couples, this work suggests that good relationships support good health and identify that sleep is sensitive to relationship quality and partner presence/absence.

Some social relationships, like pair bonds, are distinctive: two adults share a unique relationship characterized by pair-living and the hallmarks of attachment (Bales et al., 2021; Fuentes, 1998). Coppery titi monkeys (*Plecturocebus cupreus*) are pair-bonding primates that live in small social groups consisting of a single adult breeding pair and their sub-adult offspring (Mason, 1966). Relationship formation in the wild occurs when young adult titis disperse from their natal groups to find and form new social relationships and settle in their own, novel territory (Dolotovskaya et al., 2020; Mason, 1966). In the laboratory, we simulate this experience by forming new pairs in a novel enclosure. Simply moving to a novel physical environment (while holding the social group constant) results in elevated cortisol compared to baseline levels in the home environment in titi monkeys, though this elevation resolves relatively quickly (Cubicciotti et al., 1986). This change in cortisol also occurs when new pairs are formed: naïve females display elevated cortisol levels upon pairing, but returned to baseline levels within 6 days post-pairing (Hoffman, 1998; Jarcho, 2011). Interestingly, titis respond differently if they have prior pairing experience: cortisol levels in previously-paired female titi monkeys decreased after being paired with a novel male (Jarcho, 2011). Behaviorally, new pairs increase in affiliation and decrease in aggression across the first week of pairing, with the most dramatic differences occurring within the first few days (Hoffman, 1998). These results may indicate increased arousal surrounding the immediate post-pairing period, but quick attenuation as individuals adapt to their new environment. Overall, the initial post-pairing period is marked by increased cortisol and behavioral inhibition, especially by females—but these effects appear to be unique to the immediate post-pairing period.

Once a pair bond is formed, pair mates live together in a shared home territory and maintain their bond through affiliative behaviors, vocal duets, and aggression towards strangers (Fernandez-Duque et al., 1997; Mason, 1966). In the wild, titi monkey activity commences at dawn, with pair mates ranging to the borders of their territory and engaging in territorial vocal duets together (Mason, 1966). During the day, titis spend most of their time foraging for food and ranging over their territory, typically within proximity of their pair mate (Mason, 1966). Other routine behaviors that demonstrate affiliation occur throughout the day in fluctuating patterns, but are most common in the afternoon (Kinzey et al., 1977). In titis, affiliative behaviors include proximity to or contact with the pair mate, which can involve grooming, mating, and tail twining along with passive bodily contact (Fernandez-Duque et al., 1997). Tail twining is a species-specific behavior in which titi monkeys sit side by side in bodily contact or proximity and wrap their tails together and occurs between members of a family group (Moynihan, 1966). This behavior has been observed both during the day (Mason, 1966) and at night, including during sleep (Kinzey et al., 1977; Moynihan, 1966). Because affiliation is higher in the afternoon as animals settle down for the evening, we would expect performance of these affiliative behaviors to be highest at night, especially considering that titis significantly reduce locomotive activity during their sleep period (Mason, 1966). While tail twining and the accompanying bodily contact are widely considered affiliative during the day (Fernandez-Duque et al., 1997), a few studies hypothesize that these behaviors provide additional balance, thermoregulation, and postural support during sleep (Anderson, 1984; Mason, 1966; Moynihan, 1966).

To our knowledge, there are no studies on titi monkey sleep outside of postural characterizations and analysis of sleep location preferences (Kinzey & Becker, 1983; Mason, 1966; Moynihan, 1966). However, the sleep of other small-bodied primates has been quantified, which can guide our understanding of how titi monkeys might behave at night. Under 12-hour light/12-hour dark conditions and using actigraphy to examine inactivity, marmosets (*Callithrix jacchus*) slept 713-793 minutes per day in 21-52-minute bouts, tamarins (*Saguinus oedipus*) slept 707-889 minutes per day in 10-28-minute bouts, and squirrel monkeys (*Samiri sciureus*) slept 459-475 minutes per day in 9-15-minute bouts (Sri Kantha & Suzuki, 2006). For both tamarins and marmosets, these sleep durations exceed 12 hours at the upper range, indicating that at least some of the light period may be spent sleeping as well. As indicated by these findings, it is likely that titi monkeys spend much of the dark period asleep—as well as some of the light period—with short bouts of sleep characterized by brief and subtle activity between bouts. The present work bridges the gap in our understanding of titi monkey nighttime behavior and builds on existing knowledge of pair formation by investigating both affiliation and activity of new pair mates across their first week together.

Methods

Subjects and Housing

We studied 12 adult male/female pairs across the first week of a new pairing. We recorded data opportunistically from new pairs created for other research projects and colony management purposes. Before pairing, all individuals were without a pair mate for at least one week. Some individuals had no prior pairing experience ("naïve", $n = 14$) and were housed with some, or all, of their natal group (i.e., parent(s) and/or siblings) prior to pairing. Some individuals had prior pairing experience ("experienced", $n = 10$) and were housed alone for at least a week (if coming from a pair) or with their offspring (without a pair mate) prior to pairing. Subjects ranged in age from 2.02 years to 23.40 years at time of pairing, with a median age of 4.05 years at the time of pairing (*IQR* = 9.23 years). Prior pairing experience and age were related, such that animals with prior pairing experience (*Mdn* = 11.79 years, *IQR* = 4.96) were older than animals with no prior pairing experience (*Mdn* = 2.62 years, *IQR* = 1.14) at the time of pairing, $X^2_1 = 16.80$, $p < 0.001$, $d = 3.17$.

All pairs were housed in cages with dimensions of 1.2m x 1.2m x 2.1m, 1.2m x 1.2m x 1.8m, or 1.6m x 1.2m x 0.7m at the California National Primate Research Center (CNPRC).

Each subject was housed in the same cage as their pair mate once paired and all pairs were introduced and housed in a cage that was novel to both animals. The home environment was maintained at 21°C on a 12-hour light/dark cycle with lights on from approximately 06:00 to 18:00 and off from 18:00 to 06:00. This housing condition is similar to those described in previous research (Mendoza & Mason, 1986; Tardif et al., 2006). Pairs may have experienced some variation in ambient lighting due to skylights in their home room and pairings formed at different times of the year. Titi monkeys were fed monkey chow (Lab Diet, New World Primate Diet #5040), carrots, bananas, apples, and rice cereal twice daily. Water was available *ad libitum* and additional edible foraging enrichment was provided twice daily. All procedures followed NIH guidelines for the ethical treatment of laboratory animals and were approved by the University of California Davis Institutional Animal Care and Use Committee.

Behavioral Scoring

Behavioral data was collected in-person (daytime affiliation) or via video recording (nighttime affiliation and activity) for 7 days immediately following pairing, with Day 1 being the day on which pairing occurred. Video recording was obtained using an infrared-sensitive camera (Panasonic HC-VX870) and an infrared floodlight (Univivi LED IR Illuminator).

Our goal in assessing nighttime activity was to approximate behavioral sleep and our definition of activity reflects this distinction. Sleep is typically defined as a quiescent behavioral state in which an individual has reduced responsiveness to weak stimuli and rapid reversibility in response to strong stimuli (Campbell & Tobler, 1984). The first refers to the preservation of sleep in the face of potentially disruptive stimuli, differentiating sleep from wake. The second differentiates sleep from a coma or vegetative state from which an individual cannot be roused. At the macro level, the timing of sleep is regulated by circadian rhythms—approximately 24-hour cycles entrained to internal (cellular) and external (environmental) cues. For diurnal animals, this means that the wake period corresponds with the time of day when the sun is up (light period), and the sleep period corresponds with the time of day when the sun is down (dark period). The wake period is characterized by responsiveness to internal and external stimuli, voluntary motor activities, and a variety of behavioral states (Scammell et al., 2017). The sleep period is characterized by behavioral inactivity, adoption of a species-typical sleeping posture, and oscillation between non-rapid eye movement (NREM) and rapid eye movement (REM) sleep (*for review*: Anafi et al., 2019; Scammell et al., 2017). We defined activity as movement beyond gentle swaying or mild twitching. The animal did not have to be visibly awake (head up, eyes open) to be scored as active, but most often these events co-occurred.

Our assessment of affiliation between pair mates is a well-validated measure of affiliation in titi monkeys and similar data have been collected by our laboratory since 2008 (e.g., Karaskiewicz et al., 2021; Rothwell et al., 2020). All behaviors scored described the pair's relation to each other and represent conditional measures, meaning that both members of the pair needed to participate in a given behavior for it to be scored. Trained observers scored daytime affiliation (Table 1) between pair mates in-person or from video recordings every 2 hours from 06:30 to 16:30 5 days per week during the first week post-pairing (Days 1-7). Nighttime affiliation and activity were scored for all 7 days of the first week post-pairing (Days 1-7) from infrared video recordings at 22- minute intervals, yielding 30 intervals/night.

Data Analysis

For all our analyses, we calculated rates of our behaviors of interests by dividing the sum of all observations in which the behavior occurred for a given interval (e.g., nighttime on Day 1) by the total number of observations for the same interval. Affiliative behaviors were calculated both individually (e.g., tail twining) and together (i.e., sum of all tail twining, contact, and proximity) to examine differences in specific behaviors as well as overall levels of affiliation. All statistics were run in R version 4.0.5.

Both activity ($W = 0.90$, $p < 0.001$) and affiliation ($W = 0.74$, $p < 0.001$) were not normally distributed, so means comparisons were conducted using Kruskal-Wallis test with the kruskal.test() function from the stats package in base R (R Core Team, 2021). Post-hoc tests for repeated measures variables were conducted using Pairwise Wilcoxon Rank Sum Tests with multiple testing corrections with the pairwise.wilcox.test() function from the stats package in base R. Effect sizes were calculated using the cohens_d() function from the effectsize package (Ben-Shachar et al., 2020). To analyze the effects of post-pairing day on nighttime activity, we built several mixed effects models using the lmer() function from the lme4 package (Bates et al., 2015). The best-fit model, determined by comparing AICs, included a fixed effect of post-pairing day and a random effect of pair identity. To analyze the effects of post-pairing day and time of day on affiliation, we built several mixed effects models and the best-fit model included fixed effects of post-pairing day and time of day, as well as a random effect of pair identity. Degrees of freedom and p-values were estimated using the lmerTest package (Kuznetsova et al., 2017) and beta weights were obtained using the scale() function in base R.

Results

Across all days and all pairs, animals were active only in an average of 10% of all observations ($SE = 0.5\%$). Overall, nighttime activity differed between days, $X^2_6 = 14.89$, $p = 0.02$ (Table 2). Post-hoc tests revealed that nighttime activity differed only between night 1 and nights 3 ($p = 0.02$), 5 ($p = 0.04$), 6 ($p = 0.05$), and 7 ($p = 0.02$, all other ps > 0.05). In our mixed effects model, we identified a significant effect of post-pairing day on nighttime activity, $t_{(134)} = 1.99$, $p = 0.048$, $\beta = 0.16$. The intercept of this model was significant, meaning that activity was non-zero on Night 1, in which pairs were active in approximately 12% of observations ($t_{(62)} = 10.59$, $p < 0.001$, $SE = 0.01$), and activity decreased by an average of 0.5% per night across the data collection period (Figure 1). The random effect in our model, pair identity, accounted for approximately 12% of variance in our model ($\sigma = 0.12$, $SD = 0.34$) and residual variance was high ($\sigma = 0.86$, $SD = 0.93$). Of the observations in which individuals were active ($n = 438$ observations), 62% involved vigilance behaviors (e.g., observing the environment) and 25% involved postural adjustment and re-positioning. 29% of active observations were the first observations, which coincided with the automatic light switches activating and turning off. Only one experienced pair was observed engaging in mating during the dark period. Due to the sky lights in the animal rooms, we investigated how activity related to potential ambient light after the lights in the rooms turned off. Across all observations in which subjects were active, 46% occurred when the sun was up and the room lights were off. When we excluded first observations (i.e., those that coincided with the lights turning off), only 25% of active observations coincided with potential ambient light from the skylights.

Several demographic features (sex, age, and prior pairing experience) that can influence behavior in the context of titi pairings (Dolotovskaya et al., 2020; Jarcho, 2011; Rothwell et al., 2020) were not included in our best fit model. Activity rates did not differ between females ($M = 0.11$, $SE = 0.01$) and males ($M = 0.10$, $SE = 0.01$), $X^2_1 = 0.29$, $p > 0.05$, $d = 0.10$, *95% CI*: [-0.23, 0.43]. In fact, within pairs, activity was highly correlated between males and females ($r = 0.67$). Similarly, activity did not differ based on subject age at the time of pairing ($X^2_{23} = 31.41$, $p >$

0.05), and the two were not correlated (*r* = 0.11). Overall activity rates did not differ based on whether an individual animal had prior pairing experience (Figure 2): experienced individuals (*M* = 0.11, *SE* = 0.01) and naïve individuals (*M* = 0.10, *SE* = 0.01) had similar rates of nighttime activity, $X^2_1 = 3.01$, $p > 0.05$, $d = 0.33$, *95% CI*: [-0.67, 0.01].

Affiliation was high across all times and days: pairs engaged in affiliative behaviors in 73% of observations (*SE* = 3.16%). There was no difference in overall affiliation ($X^2_2 = 3.45$, $p > 0.05$), light time affiliation ($X^2_2 = 1.75$, $p > 0.05$), or dark time affiliation ($X^2_2 = 1.01$, $p > 0.05$) based on prior pairing experience. Pairs engaged in significantly more affiliation during the dark hours (*M* = 0.97, *SE* = 0.01) than during the light hours (*M* = 0.43, *SE* = 0.04), $X^2_1 = 68.19$, $p < 0.001$, $d = 2.22$, *95% CI*: [-2.66, -1.78] (Figure 3). Across post-pairing days, affiliation did not change, $X^2_6 = 11.29$, $p > 0.05$, even when separated out between light ($X^2_6 = 9.84$, $p > 0.05$) and dark ($X^2_6 = 8.42$, $p > 0.05$) observations. In our model, we found a significant effect of time of day (lights on versus lights off) ($t_{118} = 12.52$, $p < 0.001$, $\beta = 0.73$), but not post-pairing day ($t_{122} = 2.05$, $p = 0.04$, $\beta = 0.12$) on pair affiliation. When we looked specifically at different affiliative behaviors (Table 3), we found significant effects of post-pairing day ($t_{121} = 2.95$, $p = 0.004$, $\beta = 0.09$) and time of day ($t_{118} = 29.86$, $p < 0.001$, $\beta = 0.09$) on tail twining, such that tail twining generally increased across days (*estimate* = 0.02) and occurred significantly more at night (*M* = 0.93, *SE* = 0.02) than during the day (*M* = 0.12, *SE* = 0.02), $X^2_1 = 95.14$, $p < 0.001$, $d = 5.05$, *95% CI*: [-5.76, -4.34]. However, only time of day had a significant effect on contact ($t_{119} = 3.91$, $p = 0.002$, $\beta = 0.33$) and proximity ($t_{117} = 6.54$, $p = 0.002$, $\beta = 0.50$) between pair mates, such that both behaviors occurred more often during the day compared to the night.

Discussion

Nighttime activity in titi monkeys is underexplored and this work expands our understanding of nighttime behavior, especially in the context of new pair formation. Unsurprisingly, diurnal titi monkeys are not very active during the dark hours, and we observed activity in only about 10% of nighttime observations. While previous studies have quantified nighttime activity in small-bodied arboreal primates (*Callithrix jacchus, Saguinus oedipus, Samiri sciureus*: Sri Kantha & Suzuki, 2006), these metrics were combined with daytime activity and calculated over a 24h period, making it difficult to compare with nighttime-only estimates. In other laboratory species, sleep efficiency—or the time animals are thought to be asleep during the time the lights are off—varies across species. A study of three species of macaques (*Macaca mulatta, Macaca fasicularis, Macaca nemestrina*) estimated that individuals were inactive for 79.5 – 87.8% of the 12h dark period (Zhdanova et al., 2002), which indicates more nighttime activity and wakefulness compared to our sample. However, a study of two species of gibbons (*Hylobates moloch, Hylobates pileatus*) estimated that individuals were inactive in their sleeping sites as much as 98.3% of the time (Reyes et al., 2021). Across studies, activity was measured or estimated in different ways, which makes them challenging to compare. Given the scarcity of research in this area, it is difficult to contextualize our estimate of nighttime activity or compare it to estimates in other diurnal primates. Based on our knowledge of this species as territorial and vigilant (Mason, 1966), wakefulness during the dark period makes ecological sense (Sri Kantha & Suzuki, 2006). This may be particularly exacerbated by the captive environment, in which pairs are housed at a high density that is not comparable to their species-typical spatial distribution, which can affect other aspects of titi behavior including affiliation between pair mates (Lau et al., in review). Our post-hoc examination of titi behavior during “active” nighttime observations revealed that the animals were engaging in vigilance behaviors like looking around their enclosure in the majority (62%), which is consistent with the idea that titi nighttime behavior consists primarily of inactivity punctuated by brief periods of activity that reflect vigilance. 29% of the active observations coincided with the lights turning off, which is an ecologically relevant time of day. In the wild, titi monkeys settle into their sleeping sites and engage in decreased activity and increased intra-pair affiliation in the late afternoon and early evening (Kinzey et al., 1977; Kinzey & Becker, 1983). Ambient light was present in 25% of active observations after controlling for the first observation, but as the duration and intensity of ambient light experience differed by pair (i.e., date of pairing), this variable was confounded with our random effect of pair identity and was not included in our model. Even without the stimulus of the automated lighting system, it is understandable that the first (earliest) observation of the nighttime would include activity.

We expected to find some variation in nighttime activity across the first post-pairing week as individuals habituated to a new social partner and physical environment, and we observed an overall decrease in nighttime activity across the pair's first week together. While we did not find an effect of post-pairing day on overall affiliation between pair mates like previous studies (Hoffman, 1998), we did see a similar pattern of habituation within the first week in terms of activity. Affiliation plays a crucial role in pair bond formation and maintenance (Fernandez-Duque et al., 1997) and while we did not observe consistent changes across the first week, pairs did spend 73% of observations in affiliative contact. These rates were much higher at night (97%) than during the day (43%), but this makes sense in the context of titi monkey behavior and activity cycles. Daytime affiliative behaviors were more diverse than nighttime behaviors, with pairs engaging in more contact and proximity during the day compared to the night, but lower overall rates of affiliation. During the day, titi monkeys are more likely to be awake and activity levels are higher, producing a considerable amount of variability in behavior (e.g., feeding, locomotion) (Moynihan, 1966). In captivity, all animal care and most data collection occurs during the light period, which could further influence both activity and affiliation. In the wild, titi monkeys are less affiliative earlier in the day (Kinzey et al., 1977), while late afternoon and evening activity is lower and coincides with rest and sleep in affiliative contact (Moynihan, 1966). It is understandable, therefore, to see more diversity and less time spent in affiliative behaviors during the day compared to the dark hours.

Within pairs, activity was highly correlated between individuals, which is reasonable when we consider the high degree of physical contact involved in their sleeping posture. This is further supported by our findings on nighttime affiliation. Pairs spent 93% of nighttime observations tail twining, which involves immense bodily contact. It is likely that one animal awakening and becoming active would result in wake and activity of the pair mate. Previous work has identified high behavioral concordance between titi monkey pair mates during the day (Mason, 1966), which may be a factor of the closeness of their bond. This is further supported by findings in humans: nighttime behavioral concordance is associated with higher quality relationships (Gunn et al., 2015). Among titis, the sleep posture, including nighttime tail twining may serve additional purpose above and beyond affiliation. Thermoregulation may play a role in increasing the amount of time pairs spend in bodily contact with one another. Social thermoregulation is common in endothermic social animals and huddling with social others supports body heat retention, particularly as temperatures cool at night. Many other species of arboreal primates participate in bodily contact with others to maintain their body temperature (*Aotus azarae azarae*: Savagian & Fernandez-Duque, 2017; *Hapalemur meridionalis*: Eppley et al., 2017). Since primates are commonly seen to huddle for thermoregulatory purposes, it is likely that this is also why titi monkey sleeping posture includes heavy bodily contact. In the case of tail twining, it may be that pairs increase participation of this affiliative behavior for postural support (i.e., balance). Other studies have hypothesized that tail twining may provide additional balance support so as to decrease the chance of falling out of the sleeping tree (Anderson, 1984; Moynihan, 1966).

While sex, age, and prior pairing experience did not affect nighttime activity, the interconnectedness of pair mates' activity cannot be dismissed. Activity of one individual was highly correlated with that of their pair mate and titi sleeping posture may contribute to this. Just as affiliation may have different context during the day and night, different rules may govern nighttime interactions between pair mates—and their significance. Among wild titi monkeys, females control proximity more than males (Dolotovskaya et al., 2020) and previous work identified physiological differences between experienced and naïve female titis entering new pairs such that experienced females had reduced cortisol upon pairing—compared to baseline levels pre-pairing (Jarcho, 2011). Cortisol and sleep have a bidirectional relationship, such that sleep loss coincides with increased cortisol the next day (Leproult et al., 1997) and elevated cortisol associated with stress is tied to sleep loss (Mullington et al., 1996; Spiegel et al., 1999). Our results do not seem to indicate that this difference in cortisol response to pairing translates to differences in nighttime activity.

Interestingly, there was no effect of prior pairing experience on affiliation. Prior work has demonstrated that pairing tenure (Rothwell et al., 2020) can influence rates of affiliation between pair mates and new evidence found that titis that were paired after demonstrating high preference for their mate in a speed-dating paradigm were more affiliative at earlier time points than agematched controls that were quasi-randomly paired (Baxter et al., in press). While our pairing timeline was consistent across pairs, some of our naïve pairs (*n* = 6) also went through this mate choice paradigm and were paired with their preferred candidate, so it is conceivable that their affiliation was inflated relative to randomly paired naïve animals and contributed to a null difference between our groups of naïve and experienced pairs.

Limitations & Future Research

There were a few important limitations to this study. First, the presence of sky lights in our animal housing rooms introduced potential variance into our sample as different pairs may have experienced differently timed and unstandardized light cycles due to ambient light exposure. While this did not appear to have an outsized influence on our sample, it would be best to control for this in future studies. Second, while our behavioral scorers had high inter- and intra-rater reliability, behaviorally scoring activity is inherently challenging and subjective. Future studies using accelerometry to quantify activity could provide more detailed and continuous information about nighttime activity in this species, including average length of inactive bouts and duration of wake periods during the nighttime. Another limitation of this study was that nighttime and daytime observations of affiliation were conducted differently: daytime data was collected every 2 hours during the light period and scored by an in-person observer, while nighttime data was collected every 20 minutes during the dark period and scored from video recordings. Since the intervals between data points are not equal between day and night, the salience of each individual daytime observation is higher than each individual nighttime observation. We took this discrepancy into account by transforming our data into a rate, rather than a sum score. However, we may have lost sensitivity to daytime affiliation with lower numbers of observations, especially because titi monkeys are more active during the day than the night.

Our subjects for this study were paired for several projects and colony management. Because we used a convenience sample, individuals differed in prior pairing and parenting experience, testing and handling experience, and prior exposure to potential mates (e.g., speed dating). Out of our 12 pairs, 2 pairs had mixed prior pairing experience. This group was too small to run independent analyses of pair-level prior experience in the present study. Future research should explore the salience of having one naïve animal in a pair and whether there is an impact of which animal (male or female) is the naïve pair mate.

The current work focuses on quantifying nighttime activity of newly formed pairs but cannot offer explanations for titi sleeping posture or predictions about nighttime activity in established pairs. Future studies should examine physiological measures like body heat to test the thermoregulatory hypothesis of titi sleep posture and social sleep as well as examine the consequences of nighttime separation from a physiological and psychological perspective.

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Tables

Table 1. Ethogram of affiliative behaviors.

Behavior	**Definition**
Tail Twining	Animals' tails are intertwined for at least one full turn
Contact	Animals' bodies are in physical contact
Proximity	Animals are within arm's length of one another (approximately 6 inches), excluding the tail
None	Pair mates are not within proximity, in contact, or tail twining

Table 2. Average nighttime activity across the first week of pairing. Activity was calculated as the number of "active" observations divided by the total number of observations for a given subject on that day.

Day	**M**	**SE**
1	0.14	0.01
2	0.11	0.02
3	0.09	0.01
4	0.12	0.01
5	0.10	0.01
6	0.10	0.01
7	0.09	0.01

Table 3. Rates of all affiliative behaviors during the light and dark period over the first post-pairing week. Rates of behaviors were calculated as the number of observations including the behavior divided by the total number of observations for that period and day (e.g., Day 1, light period). Behaviors were scored as mutually exclusive but note that the tail twining posture involves both proximity to and contact with the pair mate. Pairs spent more time tail twining during the dark period compared to the light period ($p < 0.001$) and engaged in more tail twining across post-pairing days ($p < 0.001$).

Post-Pairing Day	**Tail Twining**				**Contact**				**Proximity**			
	Light Period		**Dark Period**		**Light Period**		**Dark Period**		**Light Period**		**Dark Period**	
	M	**SE**	**M**	**SE**	**M**	**SE**	**M**	**SE**	**M**	**SE**	**M**	**SE**
1	0.02	0.02	0.85	0.10	0.11	0.07	0.03	0.02	0.08	0.05	0.00	0.00
2	0.14	0.06	0.91	0.04	0.14	0.06	0.03	0.02	0.17	0.05	0.01	0.01
3	0.13	0.05	0.92	0.03	0.13	0.04	0.08	0.03	0.32	0.09	0.01	0.00
4	0.00	0.00	0.96	0.02	0.12	0.08	0.03	0.02	0.21	0.08	0.00	0.00
5	0.36	0.15	0.95	0.02	0.20	0.16	0.04	0.02	0.11	0.05	0.00	0.00
6	0.06	0.04	0.97	0.01	0.18	0.09	0.02	0.01	0.12	0.08	0.00	0.00
7	0.23	0.05	0.97	0.01	0.11	0.05	0.02	0.01	0.17	0.11	0.00	0.00

Figure Legends

Figure 1. Nighttime activity decreases across post-pairing days in newly formed pairs. Rate of activity for each subject is displayed as thin lines, overlaid by the mean rate of activity across days. Overall, nighttime activity decreased across the first post-pairing week.

Figure 2. Nighttime activity across post-pairing days does not differ by prior pairing experience. Naïve animals came to their first pairing from their natal group and Experienced individuals came to their pairing either from at least a week of separation from a prior partner or a non-paired social group (e.g., living with offspring). Rate of nighttime activity for each subject is displayed as thin lines, overlaid by group means.

Figure 3. Intra-pair affiliation did not change across the first post-pairing week. Nighttime ("Dark" period, 18:00-06:00) affiliation was consistently higher than daytime ("Light" period, 06:00-18:00) affiliation across the post-pairing week. Affiliation also varied more during the light period, both between pairs and in respect to the different behaviors (e.g., tail twining with, affiliative contact with, proximity to the pair mate) observed. Dark period affiliation was most often tail-twining and accompanied by low or no activity.

Figure 1

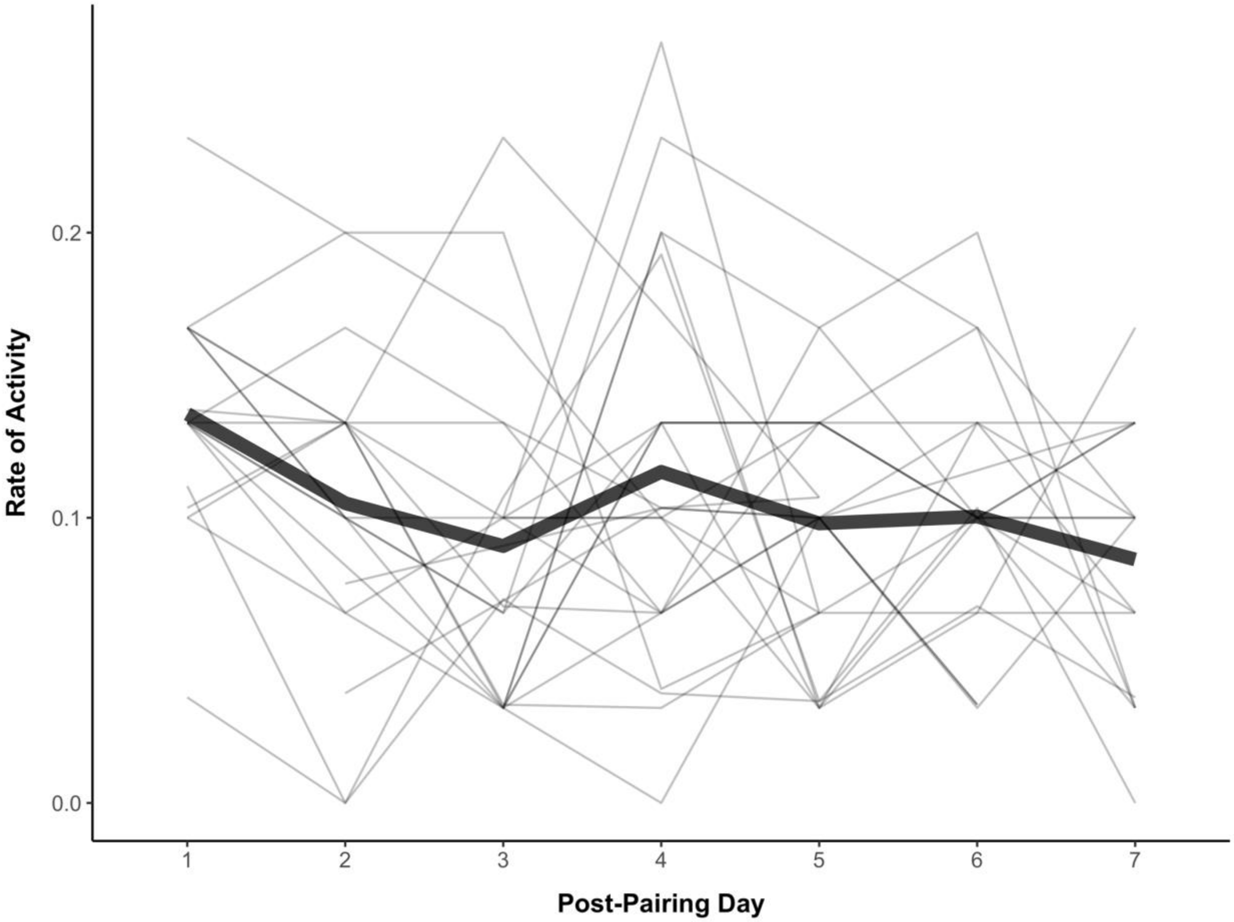


Figure 2

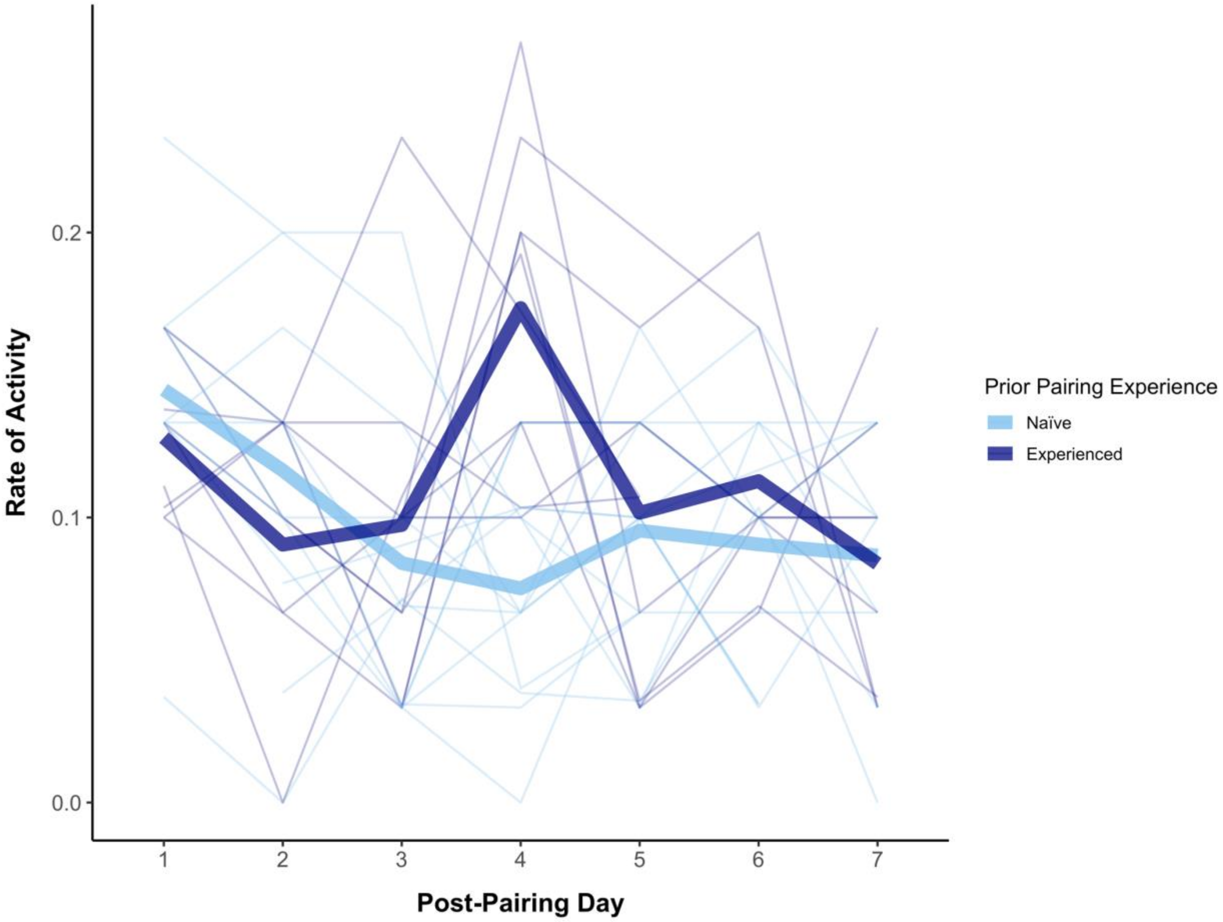
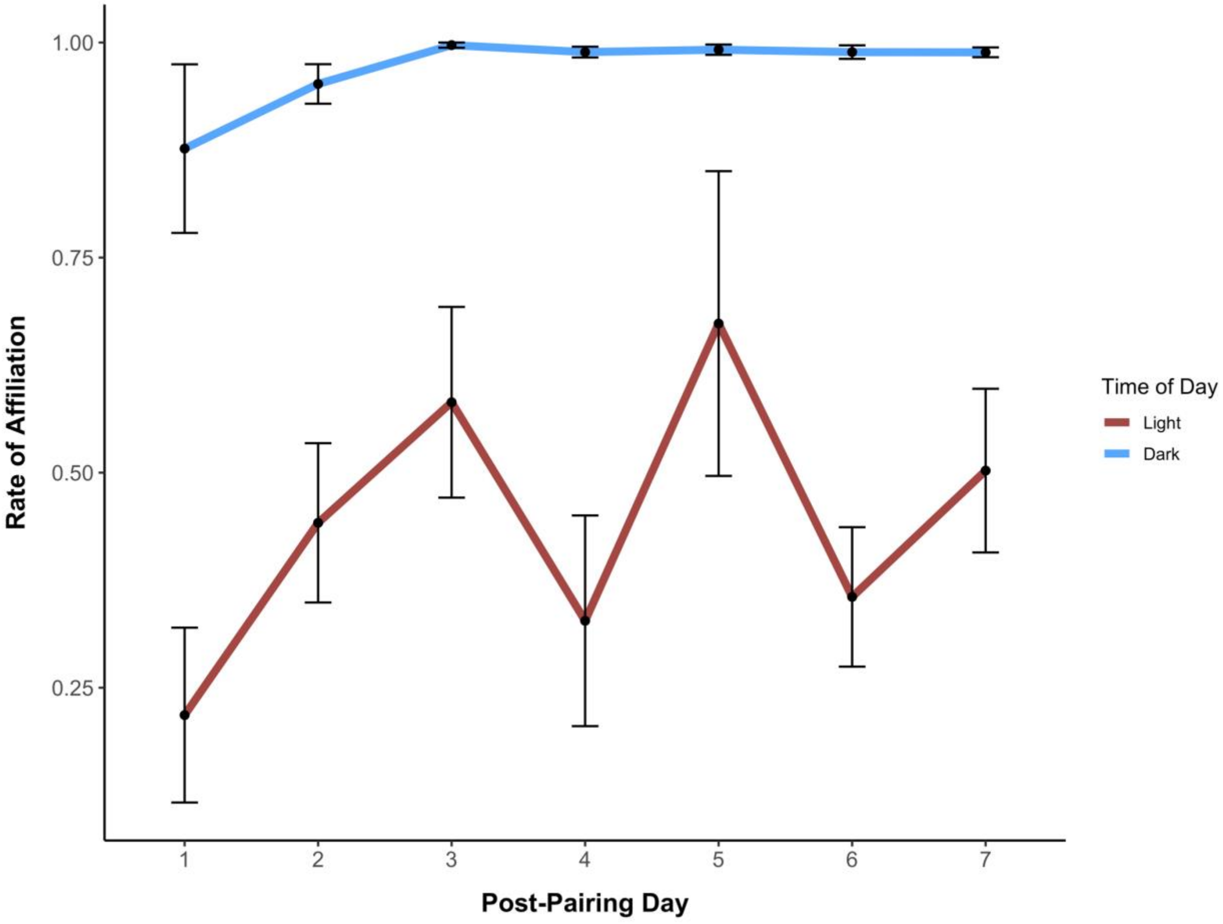

Rate of Activity
0.2
0.1
0.0
1
2
3
4
5
6
7
Post-Pairing Day
Prior Pairing Experience
Naïve
Experienced

Figure 3


Rate of Affiliation
1.00
0.75
0.50
0.25
1
2
3
4
5
6
7
Post-Pairing Day
Time of Day
Light
Dark

Chapter 2: Physiological and behavioral effects of hormonal contraceptive treatment in captive, pair-bonded primates (*Plecturocebus cupreus*)

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Abstract

Hormonal contraception is an effective, reversible tool for managing birth rates in humans and nonhuman animals alike. However, manipulating reproductive hormones has behavioral consequences that can impact social and sexual behavior between conspecifics. We studied $n = 35$ non-reproductive pairs of coppery titi monkeys (*Plecturocebus cupreus*) to ascertain whether treatment with two different forms of hormonal contraception (deslorelin acetate implants, medroxyprogesterone acetate injections) differentially influenced the pair relationship compared to untreated females paired with vasectomized males. We found no differences in rates of affiliation over a 5-month period between pairs in which the female was untreated compared to either pairs in which the female was treated with deslorelin acetate ($t_{(32)} = 0.64$, $p > 0.05$, $\beta = 0.08$), or treated with medroxyprogesterone acetate ($t_{(32)} = 0.88$, $p > 0.05$, $\beta = 0.11$). Similarly, there were no differences in affiliation between pair mates in the two treatment groups ($t_{(32)} = 0.37$, $p > 0.05$, $\beta = 0.04$). This work is the first to examine behavioral consequences of hormonal contraception in a pair-bonding species and the results are encouraging for managing captive, breeding colonies of social animals, especially those that participate in behavioral research.

Introduction

Reproduction is critical for the continuation of a species, but unchecked reproduction can have negative consequences. In animal populations, contraception is used to manage the size, impact, and genetic make-up of populations. In the wild, implementing contraceptive practices is an ethical way to manage populations that decreases the need for more extreme methods like culling (Gupta & Minhas, 2017). In captive populations, controlling reproduction is important to ensure that facilities can financially and physically care for all offspring that are born and manage population-level genetic diversity among constrained populations (Wallace et al., 2016). Hormonal contraceptives are an effective method by which to control reproduction in a research species because they are both long acting and reversible, while physiological alterations to reproductive anatomy (e.g., spaying, neutering, vasectomy, tubal ligation, or hysterectomy) are highly effective, but not usually reversible (Wallace et al., 2016). However, the introduction of hormonal contraceptives may impact behavior. In social species—particularly those that form and maintain attachment relationships to their mating partners—it is important to understand the behavioral side effects of hormonal contraceptive use.

Mating behavior and attraction can be influenced by the activity of exogenous reproductive hormones. In stump-tailed macaques (*Macaca arctoides*), females treated with Depo-Provera, a systemic progestin-based contraceptive, engaged in more subordinate behaviors (e.g., fear grimaces) and antagonistic behaviors (e.g., stare threats) toward males than did untreated females (Linn & Steklis, 1990). Hormone levels in females may also influence male mate choice. Male stump-tailed macaques did not approach or inspect the genitals of treated females as often as they did for untreated females and copulated less often with treated females (Linn & Steklis, 1990). For some species, the mechanism for distinguishing treated versus untreated females may be olfactory. Male ring-tailed lemurs (*Lemur catta*) preferred the scent of untreated females compared to the scent of females that were being treated with Depo-Provera (Crawford et al., 2011). In other species, visual cues may help males distinguish between treated and untreated females. Among hamadryas baboons (*Papio hamadryas*), a species that displays overt visual signs of ovulation, males were less likely to mate with Depo-Provera treated females compared to untreated females—even when those untreated females did not display peak fertility via full genital swellings (Guy et al., 2008). Taken together, these results suggest that hormonal contraceptive treatment can affect both attraction and social behavior of male and female nonhuman primates, possibly through hormonal pathways and sensory cues.

While some work on the impact of contraceptive use on social behavior of nonhuman primates exists, the species studied thus far employ non-monogamous mating strategies and display overt visual ovulation signals (e.g., size and/or color changes of genital region during ovulation). It is important, therefore, to also study these effects in species that employ other mating strategies in order to better understand how different social systems may be impacted by hormonal contraceptive treatment. One social system of particular interest in this area is social monogamy. Coppery titi monkeys (*Plecturocebus cupreus*) are small South American primates that form deep and lasting attachments to their pair mates (Mason, 1966). They are socially monogamous (Fuentes, 1998) and show a consistent preference for their mate, especially in the presence of a stranger (Carp et al., 2016). Pair mates groom one another, stay near each other, and twine their tails together—all of which are regarded as signs of affiliation used in pair-bond maintenance and evidence of a close, preferential relationship (Fernandez-Duque et al., 2000; Mason, 1966; Moynihan, 1966). While new pairs exhibit low affiliation immediately upon pairing (Hoffman, 1998), levels of affiliation rise within the first week and remain consistently high (Rothwell et al., 2020). Due to their close social relationships and monogamous mating strategy, titi monkeys are an ideal animal model in which to study the effect of hormonal contraception on social behavior between bonded reproductive partners.

The titi monkey reproductive cycle is like that of humans: estrogen and progesterone fluctuate based on the day and phase. During the luteal phase, estrogen increases and peaks at ovulation, while progesterone peaks in the luteal phase (Valeggia et al., 1999). The average titi ovulatory cycle is approximately 17 days and can be identified from urinary assays of estrone conjugate (E1C) and pregnanediol-3alpha-glucuronide (PdG) (Valeggia et al., 1999). Like humans, the female reproductive cycle is regulated through the hypothalamic-pituitary-gonadal (HPG) axis. The hypothalamus produces gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland to release gonadotropins: follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH then stimulate the ovaries to produce estrogens and progestagens. Together, the hormones and gonadotropins drive the phases of the reproductive cycle. Captive titi monkey reproduction can be managed using hormonal contraception, but no studies to date have examined the physiological and behavioral consequences of its use. In fact, only two papers (Conley et al., 2022; Valeggia et al., 1999) have examined the endocrinology of the titi reproductive cycle at all. Understanding how titi monkey behavior is affected by hormonal contraceptive use is important both for managing their care in captive populations and comparing our findings to human behavior in the context of monogamous relationships.

In the current study, we analyzed urine from contraceptive-treated and untreated females to confirm that the contraceptive implant used in our colony altered the female reproductive cycle. Given the aim and efficacy of contraceptive implants in other species, we hypothesized that treated females would have consistently lower levels of estrogen and progesterone conjugates compared to untreated females. Second, we wanted to know how treatment with hormonal contraception would affect the relationship between pair mates. We examined rates of affiliation between pairs in which the female was treated with a contraceptive implant, pairs in which the female was treated with a contraceptive injection, and pairs in which the female was untreated. Given evidence from other species examining social behavior following treatment with hormonal contraceptives, we hypothesized that pairs containing treated females would have lower average rates of affiliation compared to pairs containing an untreated female.

Methods

Subjects

All subjects were coppery titi monkeys (*Plecturocebus cupreus*) born and housed at the California National Primate Research Center (CNPRC). Subjects used to assess physiological effects of deslorelin were n = 18 non-reproducing female titi monkeys. Half (*n* = 9) were treated with hormonal contraceptive implants, while the other half were untreated, but paired with males that previously received a vasectomy. Subjects used to assess the effects of deslorelin and medroxyprogesterone acetate on behavior were *n* = 35 different nonreproductive titi monkey pairs. In *n* = 26 pairs, the females were treated with hormonal contraceptives and their male pair mates were unaltered (*n* = 17 deslorelin acetate implants, *n* = 9 medroxyprogesterone acetate injections). In *n* = 9 pairs, the females were untreated and their male pair mates previously received vasectomies. The subjects in this study represent a convenience sample: females were not treated with hormonal contraceptives, nor did males receive vasectomies for the express purpose of this study. Contraception in titi monkeys at the CNPRC is employed for several reasons, from project needs for ongoing experiments to colony management and medical treatment following previous birth complications or uterine abnormalities (Kanthaswamy & Bales, 2018).

All animals were housed in enclosures measuring at least 1.2 × 1.2 × 0.7 m. The environment was maintained at 21◦C on a 12-h light cycle with lights on from 06:00 to 18:00. Titi monkeys were fed monkey chow, carrots, bananas, apples, and rice cereal twice daily. Water was available *ad libitum* and additional edible foraging enrichment was provided twice daily. Subjects were housed in male-female pairs and did not conceive offspring during the project. This housing situation is the same as described in previous studies (e.g., Mendoza & Mason, 1986; Rothwell et al., 2020). All procedures followed NIH guidelines for the ethical treatment of laboratory animals and were approved by the University of California, Davis Institutional Animal Care and Use Committee.

Hormonal Contraception & Reproduction

Females that were treated with deslorelin acetate implants received one 4.7 mg Suprelorin F implant (Virbac AH, Inc., Fort Worth, Texas) that was inserted subcutaneously with a single-use syringe, 14-gauge needle at the mid-back, off-center of the midline by a trained animal health technician. No suture was needed to close the remaining skin defect following insertion. Device placement was confirmed with manual palpation. Animals received 5 mg/kg of meloxicam to manage symptoms of device insertion. Treated animals were monitored for complications and pregnancy via ultrasound every 3 months and received new implants every 6 months. Deslorelin acetate is a GnRH agonist (Figure 1B), which initially increases gonadotropin and steroid hormone production, prior to downregulating pituitary GnRH receptors (Loumaye &

Catt, 1983) and ultimately suppressing the gonadotropins and hormones necessary for reproductive cycling (Carroll et al., 2022).

Females treated with medroxyprogesterone acetate received monthly 15 mg injections (Depo-Provera, AmerisourceBergen, Conshohocken, PA) intramuscularly. The injection functions by adding exogenous progesterone to the system, which triggers the negative feedback loop and shuts down production of GnRH, FSH, and LH—thereby decreasing the body's production of estrogen and progesterone needed for reproductive cycling (Figure 1C). The efficacy of these injections is reported in a variety of species (Asa & Moresco, 2019) and reproductive hormones are suppressed similar to nonovulatory cycles (Mustoe et al., 2012).

There was no difference between groups in respect to previous reproductive experience, $F(2, 32) = 0.70$, $p > 0.05$. Pairs in which the female was untreated, and the male received a vasectomy had successfully reared the same number of offspring with their pair mate ($M = 2.33$, $SE = 1.26$) as females treated with medroxyprogesterone acetate ($M = 1.11$, $SE = 0.11$) and females treated with deslorelin acetate ($M = 1.59$, $SE = 0.39$).

Urine collection & analysis

To confirm that our deslorelin-treated females were not cycling, we obtained 12 urine samples for each female. Samples spanned 24–25 days and were collected on Mondays, Wednesdays, and Fridays during the collection period. A urine analysis was run on each sample to obtain estrogen (E1C) and pregnanediol (PdG) levels. Luteal phases were defined as PdG concentrations that exceeded 100 ng/mg Cr in two consecutive samples, which together exceeded 400 ng/mg Cr and ovulation was assumed if these conditions were met (Conley et al., 2022).

Urine collection methods were similar to those described in past studies (e.g., Witczak et al., 2021). Experimenters entered subjects' home cages prior to lights-on (approximately 06:00) and waited until the subject naturally urinated. Urine was caught free-fall in a collection cup and taken back to the laboratory, where it was aliquoted and frozen at -80℃ until assay. Subjects were habituated to urine collection procedures, which have occurred multiple times weekly in the colony since 2005. This collection represented the first void urine, but not a 24-hour summed urine collection.

Assays for E1C and PdG were conducted as previously described (Conley et al., 2022; Valeggia et al., 1999) in the Clinical Endocrinology Laboratory at the University of California, Davis. Briefly, urine was diluted in water (E1C 1:200, PdG 1:4) to ensure determinations were within the range of the standard curves for each analyte. Horseradish peroxidase conjugated to E1C (1: 240,000 dilution) or PdG (1:150,000 dilution) was added, plates were thoroughly mixed and incubated at 4℃ overnight. The following morning, plates are washed 4 times in wash solution, then 100 µL of freshly prepared substrate solution (0.05 M citrate, pH 4.0, 0.4 mM ABTS, 1.6 mM H2O2) was added. Plates were read when the average optical density of the total binding wells was at an absorbance of 1.0. Intra- and inter-assay coefficients of variation were < 17% for both E1C and PdG assays.

Behavioral Data

Behavioral data was recorded up to 6 times daily in two-hour intervals during daytime hours (6:30 to 16:30) for 5 days a week using a scan sampling technique (Altmann, 1974). For each observation a trained observer recorded the social behavior between pair mates from an existing ethogram. These data have been collected since 2008 in our laboratory and are frequently used as a measure of intra-pair affiliation (e.g., Karaskiewicz et al., 2021). Pair affiliation was recorded for the full 5-month observation period described above. Every two hours, a trained observer recorded whether adult partners were within physical proximity (Proximity), social contact (Contact), tail twining (Tail Twining), or none of the above (None). The frequency of these behaviors was recorded across 5 consecutive months (20 weeks) of contraceptive use. For our analyses, social affiliation was measured as the proportion of time a pair spent in proximity, contact, or tail-twining—as opposed to none of these behaviors—out of all the observations collected on that pair over a particular period. This method has been previously used to summarize longitudinal affiliation in titi monkeys using similar data (Karaskiewicz et al., 2021; Witczak et al., 2022). Pairs were observed an average of 399.49 times (*SE* = 24.65, *range* = 98–536), with variation in the number of observations per pair attributable to the research hiatus caused by the COVID-19 pandemic in 2020.

Data Analysis

All statistics were performed in R (R Core Team, 2021) with a significance level set at $\alpha = 0.05$. To determine whether females treated with deslorelin acetate implants were cycling, we calculated mean values for each female, and then compared the levels of E1C and PdG between treatment groups. As both E1C ($W = 0.71$, $p < 0.001$) and PdG ($W = 0.54$, $p < 0.001$) were non-normally distributed, we compared hormones between groups using Kruskal-Wallis tests using the kruskal.test() function in base R.

To investigate the relationship between hormonal contraceptive treatment and affiliation within pairs of titi monkeys, we compared affiliation over a 5-month period between non-reproductive pairs in which the female was treated with two different forms of hormonal contraceptive and pairs in which the female was untreated. To calculate rates of affiliation within a pair, we condensed daily behavioral observations into weekly means of affiliation (sum of all tail twining, contact, and proximity observations/total number of observations).

To examine changes in the rate of pair mate affiliation between groups, we employed mixed-effects models using the lmer() function from the lme4 package (Bates et al., 2015). Degrees of freedom and p-values were estimated using the lmerTest() package (Kuznetsova et al., 2017), which estimates significance using Satterthwaite approximations. Our model included the fixed effect of contraceptive method (levels: deslorelin acetate, medroxyprogesterone acetate, vasectomy) and a random effect of pair identity. In our sample, pair identity encompasses all variance attributable to the pair—such as pairing tenure, duration of contraceptive use, previous pairing experience, previous parenting experience, and subject age. Models that accounted for these factors fit the data less well and were confounded by the inclusion of the random effect. Therefore, the random factor was included in the final models, but not pairing tenure, duration of contraceptive use, previous pairing or parenting experience, and subject age. Effect sizes were calculated using the cohens_d() function from the effectsize package (Ben-Shachar et al., 2020).

Results

Physiological effects of deslorelin

In this study, we investigated the behavioral consequences of hormonal contraceptive treatment in a managed captive population of titi monkeys. First, we wanted to confirm the physiological effects of deslorelin acetate implants in titi monkeys as a prelude to more substantive questions about behavior. To do this, we compared reproductive hormone levels between pairs in which the female was treated with deslorelin and pairs in which the female was untreated. Overall, our data span 24 days of reproductive cycling, which encompasses at least one full reproductive cycle in this species (Valeggia et al., 1999). As predicted, treated females (*Mdn* = 201.10, *IQR* = 112.50) had significantly lower levels of urinary estrogens compared to untreated females (*Mdn* = 690.10, *IQR* = 649.83, $X^2(1) = 123.65$, $p < 0.001$, $d = 1.35$, *95% CI* [-1.65, -1.06]). Similarly, treated females (*Mdn* = 13.95, *IQR* = 10.20) had significantly lower levels of urinary progestagens compared to untreated females (*Mdn* = 53.20, *IQR* = 144.70, $X^2(1) = 81.38$, $p < 0.001$, $d = 0.93$, *95% CI* [-1.21, -0.65]). While all our treated females failed to meet criteria to be considered cycling, some of our untreated females ($n = 4$) were also not cycling (Figure 2). Cycling females had higher levels of both E1C ($t_{(2)} = 9.30$, $p < .001$, $\beta = 0.62$) and PdG ($t_{(2)} = 10.41$, $p < .001$, $\beta = 0.68$) compared to treated non-cycling females (Figure 3). Compared to untreated non-cycling females, untreated cycling females had similar levels of E1C ($t_{(2)} = 1.63$, $p > 0.05$, $\beta = 0.11$) but higher levels of PdG ($t_{(2)} = 7.36$, $p < 0.001$, $\beta = 0.48$). Among non-cycling females, untreated females had higher levels of E1C ($t_{(2)} = 6.82$, $p < 0.001$, $\beta = 0.49$), but similar levels of PdG ($t_{(2)} = 1.45$, $p > 0.05$, $\beta = 0.10$) compared to treated non-cycling females.

Behavioral effects of contraception

Our main aim of this study was to determine whether hormonal contraceptive use (deslorelin or medroxyprogesterone acetate) altered behavior between pair mates in our colony. To do this, we compared rates of affiliation between pairs in which the female was treated with hormonal contraceptives and pairs in which the female was untreated. In total, our data represent a mean of 149.03 days of data per pair (*SE* = -0.29, *range* = 142–150). Overall, pairs spent an average of 27% of observations in affiliative contact (*SE* = 1%).

We assessed several demographic features of individuals and pairs to identify potentially influential confounding variables. Neither female age ($F(2, 32) = 0.60$, $p > 0.05$) nor male age ($F(2, 32) = 0.08$, $p > 0.05$) differed between groups (Table 1). Similarly, pair tenure—time between pair introduction and collection of behavioral data—did not differ between groups ($F(2, 32) = 0.17$, $p > 0.05$). Contraceptive tenure—time between contraception start and collection of behavioral data—differed between groups ($F(2, 32) = 3.79$, $p = 0.03$). Specifically, pairs in which the male had a vasectomy had longer contraceptive tenures compared to pairs in which the females were treated with deslorelin ($t_{(32)} = 2.59$, $p = 0.01$, $\beta = 0.50$) and pairs in which the females were treated with medroxyprogesterone ($t_{(32)} = 2.24$, $p = 0.03$, $\beta = 0.43$) (Table 2).

When we examined the rate of intra-pair affiliation between contraception groups, we employed the best fit linear fixed-effects model described above, which included a fixed effect of contraceptive method and a random effect of pair identity. We found no significant effect of contraceptive method on the rate of affiliation between pair mates (Figure 4). There was no difference in affiliation between pairs in which the female was untreated ($M = 0.31$, $SE = 0.01$) and either pairs in which the female was treated with deslorelin acetate ($M = 0.27$, $SE = 0.01$, $t_{(32)} = 0.64$, $p > 0.05$, $\beta = 0.08$) or pairs in which the female was treated with medroxyprogesterone acetate ($M = 0.26$, $SE = 0.01$, $t_{(32)} = 0.88$, $p > 0.05$, $\beta = 0.11$). Neither was there a difference in affiliation between the two groups in which the females were treated with hormonal contraception ($t_{(32)} = 0.37$, $p > 0.05$, $\beta = 0.04$). In reference to the random effect, pair identity accounted for only about 2% of variation in affiliation across groups ($\sigma^2 = 0.02$, $SD = 0.12$). Residual variance indicated that only 3% of within-group variance is explained by differences between pairs ($\sigma^2 = 0.03$, $SD = 0.17$).

Discussion

We wanted to know how contraceptive use affects the behavior of titi monkeys, specifically whether treatment with various forms of hormonal contraceptives influenced affiliation between pair mates. Before investigating behavior, we confirmed that our newest method of hormonal contraception altered females' reproductive hormones. Deslorelin acetate implants have only recently been used for contraception in our colony of titi monkeys and the physiological effects had not yet been investigated. Consistent with work in other primates (Carroll et al., 2022), we found that females treated with contraceptive implants had lower overall urinary levels of estrogen and pregnanediol compared to untreated females. This finding serves as confirmation that the deslorelin-treated females in our sample demonstrated reproductive suppression as intended.

In respect to the relationship between behavior and hormonal contraception, we found no effect of contraceptive treatment on affiliation between pair mates. Our results contrast with findings in other species of primates, which found lower male engagement with females treated with medroxyprogesterone acetate (Crawford et al., 2011; Guy et al., 2008; Linn & Steklis, 1990). However, there are several relevant species-specific and design factors to consider. First, females tend to be the individuals to regulate affiliative contact with their mates in titi monkeys (Dolotovskaya et al., 2020), so male-driven interest may play a lesser role in titis compared to other species. Second, titi monkeys appear to have visually-concealed ovulation, meaning that they do not display visual signal of ovulation—at least that humans can perceive. Some previous work on behavior and contraceptive treatment has included non-visual stimuli (e.g., olfactory stimuli: Crawford et al., 2011), but most stimuli are either visual-specific or unspecific (i.e., live social interactions in which determining the mechanism by which conspecifics differentiate between cycling versus non-cycling females is unclear). While ovulation concealed from human perception may not be concealed from conspecifics, the other primate species studied in this area display overt visual signals of ovulation (Guy et al., 2008; Linn & Steklis, 1990). Such overt ovulation signaling often coincides with mating strategy: females in non-monogamous species, specifically those with multi-male social groups, typically display exaggerated genital swellings (Nunn & Samson, 2018). While several hypotheses may explain mating strategies in these species, most agree that paternal certainty—or lack thereof—is an important factor (Nunn & Samson, 2018). Monogamous species have greater paternal certainty by virtue of their breeding strategy, which may alter mating strategy, social behavior, and the important of timed copulation. Finally, our measure of affiliation was conditional, meaning that both members of the pair needed to engage in the behavior for it to be scored. Because we did not record approaches and their acceptance or rejection, it is difficult to know whether a null effect in our sample reflects unaltered male behavior or female compensation for lowered male interest.

To examine the impact of individual differences on affiliation between pair mates, our model also included a random effect of pair identity. We found that individual differences between pairs accounted for only 2% of variation in affiliation across all pairs, and only 3% of within-group variance could be explained by differences between pairs. This relatively small inter-pair variation may be explained by the similarities between our subjects in terms of age and pair length, as previous work has identified these as salient factors in affiliation between titi pair mates (Rothwell et al., 2020). Additional sources of variance in affiliation between pairs in previous studies may be attributable to reproductive status, which was controlled for in this study. Among titi monkeys, pair bonds are sensitive to reproductive status. Males that reproduced with their female pair mate (“fathers”) showed different neural responses to reunion with their pair mate than did non-fathers (Hinde et al., 2016). Furthermore, titi pairs show reduced rates of affiliation while engaging in active parenting (e.g., before and during pregnancy), an effect which is sustained even after the offspring are largely independent (Karaskiewicz et al., 2021). These findings motivated our decision to examine affiliation only in non-reproductive pairs, especially since pair bonds in titi monkeys appear to be resilient to reproductive failure.

Limitations & Future Directions

There were a few key limitations to this study. As discussed, we evaluated affiliation using conditional measures: both the male and female had to be participating in the behavior for it to be scored. In this way, it is difficult to know whether the male or female drove the patterns of affiliation. Though we can hypothesize based on prior research in this area, we cannot truly identify which animal steered affiliative interactions. Future research should focus on more nuanced behavior, such as approach acceptance and rejection, to identify sex-specific changes in behavior. Second, because this was a convenience sample, we had relatively little control over the timing of current contraceptive treatment or prior treatment. Our deslorelin acetate-treated group was almost double the size of our other groups as our colony is primarily managed with this type of contraception. Finally, while we only looked at affiliation between pair mates, other studies have observed increased aggression and submission in hormonally contraceptive-treated females compared to untreated females (Linn & Steklis, 1990). It would be useful to look at aggression between pair mates as well as within the family unit for contracepting pairs that are also rearing offspring to better manage a captive, contracepting population.

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Tables

Table 1. Subject age (days) by sex and contraceptive treatment group. There were no significant differences in female or male age between groups.

	Female		Male	
	M	SE	M	SE
Vasectomy	2799.00	819.26	3423.67	751.27
Deslorelin acetate	3217.00	405.94	3121.94	562.03
Medroxyprogesterone acetate	2418.44	385.41	3407.56	566.47

Table 2. Pair and contraceptive tenure (days) by contraceptive treatment group. Tenure is in relation to the first day of affiliation data collection. Negative numbers indicate treatment that began prior to affiliative behavior observations.

	Pair Tenure		Contraceptive Tenure	
	M	SE	M	SE
Vasectomy	841.78	386.38	73.67	44.52
Deslorelin acetate	1027.94	162.01	1.94	1.94
Medroxyprogesterone acetate	961.00	143.17	2.67	2.67

Figure Legends

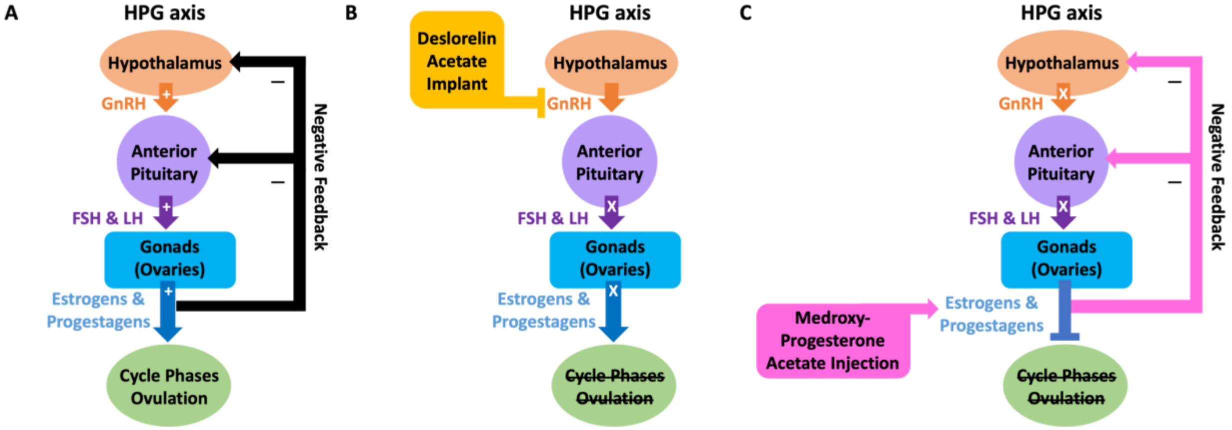
Figure 1. **Physiological mechanisms of the ovulatory cycle and hormonal contraception.** (A) The HPG axis as it typically functions. (B) The HPG axis as it functions with the addition of a deslorelin acetate implant, which results in the cessation of cycling through GnRH agonism and the subsequent inhibition of HPG function. (C) The HPG axis as it functions with the addition of a medroxyprogesterone acetate injection, which results in the cessation of cycling through the addition of exogenous progesterone, which triggers the negative feedback loop and inhibits HPG function.

Figure 2. Examples of hormone patterns across treatments. Some untreated females were cycling (left panel), while some untreated females were not cycling (center panel). All treated females were not cycling (right panel).

Figure 3. Hormone levels in females by treatment and cycling status. E1C (A) and PdG (B) levels in females that were treated with deslorelin acetate implants and not cycling (n = 9), untreated females paired with vasectomized males that were not cycling (n = 4), and untreated females that were cycling (n = 5). In reference to estrogen (E1C), there was a difference between treated and untreated females such that treated females had lower levels of E1C compared to both cycling and non-cycling untreated females. In reference to pregnanediol (PdG), there was a difference between cycling and non-cycling females such that cycling females had higher levels of PdG compared to both treated and untreated non-cycling females.

Figure 4. **Distribution of affiliative rates between pair mates in each contraceptive treatment group.** Untreated females paired with males that received vasectomies (white), females treated with medroxyprogesterone acetate injections (light grey), and females treated with deslorelin acetate implants (dark grey). Mean rate of affiliation for each pair is overlaid within their respective groups. Rates were consistent between all three groups.

Figure 1


A
HPG axis
Hypothalamus
GnRH
+
Anterior Pituitary
FSH & LH
+
Gonads (Ovaries)
Estrogens & Progestagens
+
Cycle Phases Ovulation
–
–
Negative Feedback
B
Deslorelin Acetate Implant
HPG axis
Hypothalamus
GnRH
Anterior Pituitary
FSH & LH
X
Gonads (Ovaries)
Estrogens & Progestagens
X
~~Cycle Phases~~ ~~Ovulation~~
C
HPG axis
Hypothalamus
GnRH
X
Anterior Pituitary
FSH & LH
X
Gonads (Ovaries)
Medroxy-Progesterone Acetate Injection
Estrogens & Progestagens
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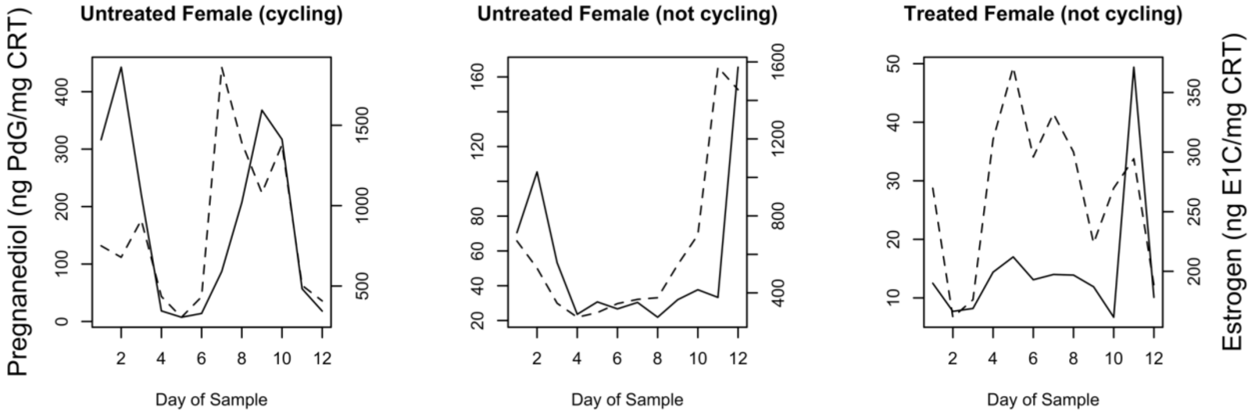
Negative Feedback

Figure 2

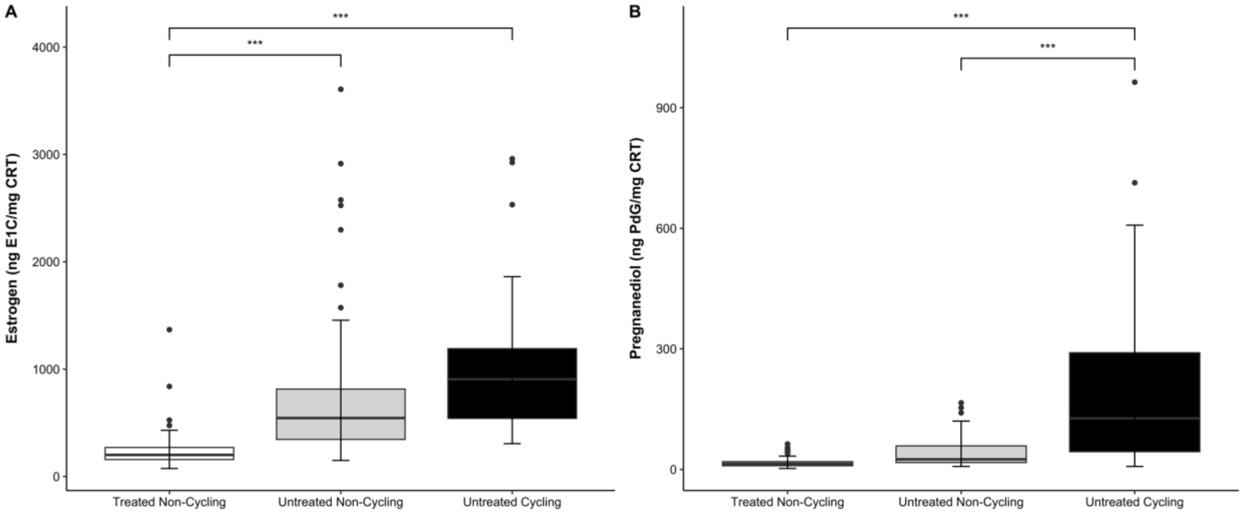


Untreated Female (cycling)

Untreated Female (not cycling)

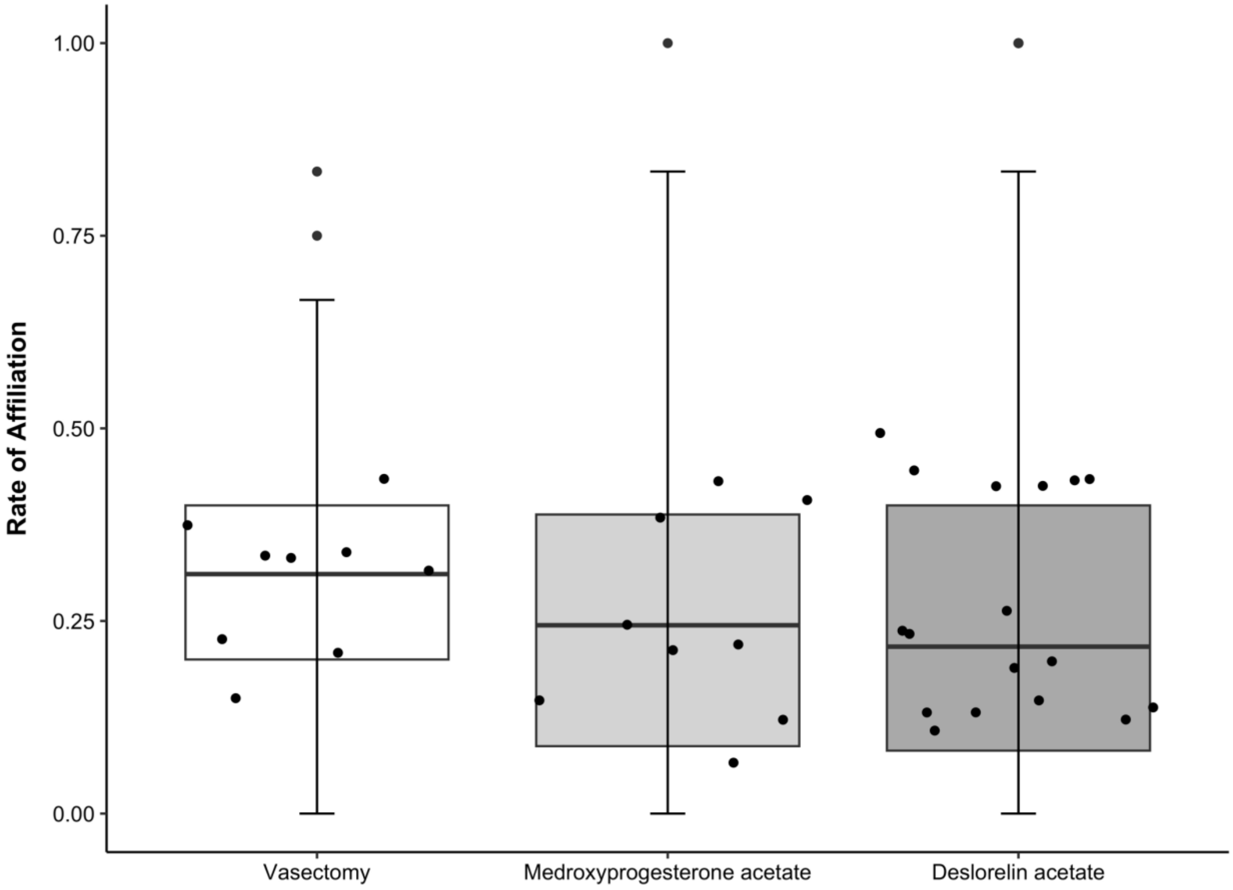
Treated Female (not cycling)

Figure 3



A
***
***
4000
3000
2000
1000
0
Estrogen (ng E1C/mg CRT)
Treated Non-Cycling
Untreated Non-Cycling
Untreated Cycling
B
***
***
900
600
300
0
Pregnanediol (ng PdG/mg CRT)
Treated Non-Cycling
Untreated Non-Cycling
Untreated Cycling

Figure 4


Rate of Affiliation
1.00
0.75
0.50
0.25
0.00
Vasectomy
Medroxyprogesterone acetate
Deslorelin acetate

Chapter 3: Parenting costs time: Changes in pair bond maintenance across pregnancy and infant rearing in a monogamous primate (*Plecturocebus cupreus*)

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Abstract

Relationships support social animals' health, but maintaining relationships is challenging. When transitioning to parenthood, new parents balance pair-bond maintenance with infant care. We studied pair-bond maintenance via affiliation in 22 adult titi monkey pairs (*Plecturocebus cupreus*) for 16 months centered around their first offspring's birth. Pair affiliation peaked during pregnancy, decreased across the postpartum period, and rose after reaching minimum affiliation 32.6 weeks postpartum. Pairs in which fathers carry infants more than average had lower affiliation at the infant's birth and return to an increase in affiliation sooner. Parents of infants who were slow to independence had higher rates of affiliation. Titi monkey infants actively prefer their fathers; mothers may avoid their infant-carrying mate, suggesting infants play an active role in parental affiliative decline. Our data supports previous findings that affiliation between partners declines following an infant's birth but demonstrates new knowledge about the extent and duration of affiliative decline.

Key words: *Pair bond, nonhuman primate, relationship maintenance, attachment, infant development, affiliation, parental care*

Introduction

Relationships are vital to the survival of social species. While social bonds form between a variety of dyadic types, adult romantic relationships—sometimes referred to as adult attachments—are some of the most potent and important bonds individuals can form (Fraley, 2019). In humans, the existence and quality of adult romantic relationships reliably predict health, happiness, and longevity (Loving & Slatcher, 2013). Individuals in long-term romantic relationships live longer (Lawrence et al., 2019), recover faster from illness or surgery (Kiecolt-Glaser, 2018), and are overall more satisfied with life (Roberson et al., 2018) than individuals who are not in romantic relationships. However, in order for a relationship to survive and thrive, a pair must engage in active pair-bond maintenance as they navigate the changes that inevitably occur across an individual's lifetime.

Just as an individual is reshaped by a myriad of life events, relationships also change over time, responding to both discrete events and continuous change. One of the earliest and most easily identified transitions is the shift from relationship formation to relationship maintenance (Clark et al., 2019). This considerable milestone attracts much attention in the current relationship science literature as specific neurobiological and behavioral changes mark this transition (Walum & Young, 2018). Both in the human and nonhuman animal literature, individuals reinforce relationships through maintenance behaviors like proximity, affiliation, shared tasks, and pair communication (*humans*: Stafford, 2016; Ogolsky & Bowers, 2013; *nonhuman animals*: Dolotovskaya, Walker, et al., 2020; Singletary & Tecot, 2020). Though the exact behaviors involved in pair-bond maintenance vary depending upon species, the types of behaviors are consistent across taxa. Further, these maintenance behaviors are particularly important not just for regular maintenance of the bond, but also when pairs experience stress or changes to their relationship. Employment of pair-bond maintenance behaviors help ensure the resilience of the bond when energetic and behavioral priorities need to shift, as is the case when a pair becomes parents.

In the transition to parenthood, romantic partners experience large scale changes in their social landscape, which alter their behavior within the relationship. Because infants of many species cannot adequately meet their own thermoregulatory, nutritional, or psychological needs, early caregivers often act as external homeostatic regulators (Hofer, 1994). Parental responsiveness supports secure infant attachment and infants can solicit parental care through vocal and behavioral solicitations (Bell & Ainsworth, 1972). In order to provide this care, new parents experience dramatic changes to their neurobiology and behavior which support infant responsivity and care (for review: Rogers & Bales, 2019). Lactation and infant carrying are the two most costly forms of infant care among mammals (Altmann & Samuels, 1992) and new parents must re-allocate energetic resources toward infant care and energetic maximization or conservation. Sometimes, this shift in energetic resource allocation results in reductions to the frequency of pair-focused social behaviors (Altmann, 1980), though there is an increasing amount of evidence to suggest that social behaviors are conserved as long as possible (*titi monkeys*: Dolotovskaya & Heymann, 2020; *black howler monkeys*: Dias et al., 2011; *gelada baboons*: Dunbar & Dunbar, 1988). As a consequence of allocating less time to social behaviors--including pair maintenance behaviors--romantic dyads may experience postpartum dips in relationship quality and satisfaction (*humans*: Belsky et al., 1985). Indeed, navigating the new balance required to meet the needs of the infant, the partner, and the self can cause considerable conflict between romantic partners (Adamsons, 2013; Cowan & Cowan, 1992). While these effects have been studied in the immediate postpartum period, most studies do not evaluate their longevity or what milestones in infant development coincide with changes in the pair relationship.

The current study longitudinally examines the relationship between pair-bond maintenance and infant care as new parents balance the needs of their relationship with the needs of dependent offspring. To investigate whether, and how, pair-focused behaviors change across the time when the dyad cares for their infant, we worked with coppery titi monkeys (*Plecturocebus cupreus*): small-bodied, socially monogamous neotropical primates that form socially monogamous pair bonds (Cubicciotti & Mason, 1976; Dolotovskaya, Roos, & Heymann, 2020; Fuentes, 1998; Kleiman, 1977; Mason, 1966) and provide biparental care to their offspring (Fragaszy, Schwarz, & Shimosaka, 1982; Mason, 1966; Mendoza & Mason, 1986). Pair relationships are maintained with affiliative behaviors (Fernandez-Duque et al., 2000), territorial mate guarding (Mendoza & Mason, 1986), and vocal duets (Lau, Clink, & Bales, 2020; Robinson, 1979). Pairs that have been together longer tend to engage in affiliative behavior more often (Hoffman, 1998; Rothwell, Carp, Savidge, Mendoza, & Bales, 2020). In addition to forming pair bonds, titi monkeys provide biparental care. Males serve as the infant's primary attachment figure and provide the bulk of non-nutritional parental care (Fragaszy et al., 1982). Females provide sustenance via lactation, carry the infant during nursing bouts, and have been observed to actively avoid and reject carrying the infant at other times (Mendoza & Mason, 1986; Reeder, 2001). Titi monkey infants actively solicit and transfer between parents, effectively shaping the frequency with which it is carried by each parent (Mendoza & Mason, 1986).

Thus far, no studies to date have examined the direct impacts of infant care on pair affiliation in a controlled manner. Here, we assess the impacts of the transition to parenthood on intra-pair affiliation. The current study tracks pair affiliation in the eight months prior to the birth of a pair's first infant and across the eight months following the birth to the first surviving offspring in an effort to uncover how and when affiliative social behaviors between pair mates change during this time. Overall, we expected pair affiliation to decline following the birth of the infant and recover as the infant becomes more and more independent. As titi monkey fathers are the primary caregivers of their infants, and mothers may avoid her partner when he is carrying the infant, we expected the proportion of time the father carries the infant to negatively predict the proportion of time the pair spends in affiliative contact. As affiliation may increase with pair tenure, we expected pair tenure to positively predict the rate of affiliation between pair mates. Finally, as infant care decreases with growing infant independence, we expected infant independence to positively predict pair affiliation.

Methods

Selection of Subjects and Housing

We identified 22 pairs of adult coppery titi monkeys (*Plecturocebus cupreus*; hereafter referred to as titi monkeys) for our study. We chose pairs for whom we had collected scan sample data when they gave birth to their first surviving infant. This criterion excluded any pairs with a first parturition prior to 2008 or after 2019. The infants of all 22 pairs survived past eight months of age. Each focal family consisted of one adult male, one adult female, and their infant. Adult females in this study ranged from 2.17 to 12.81 years of age at the time of the infant's birth (M = 4.97, SE = 0.60). Adult males ranged from 2.41 to 10.96 years of age at the time of the infant's birth (M = 5.25, SE = 0.45). The duration of pair tenure, prior to the infant's birth ranged from 0.37 to 3.57 years (M = 0.97, SE = 0.16).

All coppery titi monkeys in this study were born and housed at the California National Primate Research Center (CNPRC). Titi monkeys were housed in pairs in enclosures measuring either 1.2m x 1.2m x 2.1m, 1.2m x 1.2m x 1.8m, or 1.6m x 1.2m x 0.7m. The environment was maintained at 21 degrees Celsius on a 12-hour light cycle with lights on from 06:00 to 18:00. Titi monkeys were fed monkey chow, carrots, bananas, apples, and rice cereal twice daily. Water was available *ad libitum* and additional edible foraging enrichment was provided twice daily. Subjects were housed in male-female pairs. Subject pairs were recruited eight months prior to the birth of their first infant. After the birth of their infant, the family (male, female, and infant) remained together in the same enclosure for at least eight months. This housing situation is the same as described in previous studies (Mendoza & Mason, 1986; Tardif et al., 2006). All procedures followed NIH guidelines for the ethical treatment of laboratory animals and were approved by the University of California Davis Institutional Animal Care and Use Committee.

Data Collection

The data collected for this study included pair affiliation and parental care behavioral observations for each focal family unit. Each observation type was recorded five days a week in two-hour intervals during daytime hours (6:30 to 16:30) for a total of 16 months centered around the birth of each pair's first surviving infant. These data have been collected since 2008 in our laboratory.

Pair affiliation was recorded for the full 16-month observation period described above. Every two hours, a trained observer recorded whether adult partners were within social proximity of each other, social contact, tail-twining, or none of the above (Table 1). These measures do not include interactions with offspring. For our analyses, social affiliation was measured as the proportion of time a pair spent in proximity, contact, or tail-twining—as opposed to none of these behaviors—out of all of the observations collected on that pair over a particular period of time.

Parental care was recorded for the eight-month period following the birth of the pair's first surviving offspring. Every two hours, a trained observer recorded whether infants were being carried by their father, their mother, or neither ("off") (Table 1). This measure does not include interactions between pair mates. For our analyses, parental care was measured as the proportion of time each parent spent carrying the infant out of all of the observations collected on that infant over a seven-day period of time. Parental care was separated into the proportion of time the father carried the infant, the proportion of time the mother carried the infant, and the proportion of time either parent carried the infant.

We conducted analyses on two sets of data. The first set of analyses examined social affiliation data over a 16-month period, which began eight months before the infant was born, and ended eight months after the infant was born for each pair. Because pair tenure ranged from 0.37 to 3.57 years, not all pairs had the full eight months of data preceding their infant's birth. This happened if the pairs became pregnant immediately after pairing. Of the 22 pairs in this study, 11 pairs had the full set of eight months pre-birth data, 5 pairs had seven of the eight months of pre-birth data, and 6 pairs had five of the eight months pre-birth data. The aim of this first set of analyses was to examine broad changes in social affiliation centered around the birth of the infant. We chose to condense these data into 4 four-month periods in order to examine pair behavior during several key stages: pre-pregnancy (8 to 5 months prior to infant birth), pregnancy (4 to 0 months prior to infant birth), infant dependence (0 to 4 months postpartum), and infant independence (4 to 8 months postpartum). We chose four-month periods for two reasons: 1) pregnancy in titi monkeys is approximately four months (Valeggia, Mendoza, Fernandez-Duque, Mason, & Lasley, 1999), and 2) infants tend to transition to greater behavioral independence after four months of age (Fragaszy et al., 1982). Therefore, our four periods capture the four months directly preceding pregnancy, the entire four months of pregnancy, the first four months of an infant's life when it is highly dependent on parents, and the next four months of an infant's life when it is more independent. The proportion of time pairs spent in affiliative contact was calculated by adding the number of scan samples when a pair was in affiliative contact (tail-twining, contact, proximity) over each four-month period, and dividing by the total number of scan samples recorded during that interval. This gave us 4 four-month period social affiliation scores, with possible values ranging from 0 to 1.

The second set of analyses examined the first eight months after the infant was born with the goal of investigating the effects of parental behavior on social affiliation between partners. We used the pair affiliation scan sample data described above to measure changes in pair affiliation over time in conjunction with the parental care scan sampling described above. While infants are typically observed being carried by one parent during nearly every observation throughout the first four months of their life, the timing of the transition to behavioral independence is variable and typically happens rapidly. We considered an infant "independent" when it was observed "off" for ten consecutive observations. For our sample, mean infant independence occurred at 150.75 (SE = 4.81) days post-birth, ranging from 116 to 193 days. Because this change can occur in a matter of days, we chose to group our data by week. Therefore, we calculated the proportion of time spent in affiliative contact by adding up all of the times a pair was in affiliative contact (tail-twining, contact, proximity) over seven days, and divided by the total number of scan samples recorded during that interval. This gave us a weekly social affiliation score, with possible values ranging from 0 to 1. We then calculated three infant-carry scores: father carry, mother carry, and parent carry. Father carry was calculated by dividing the number of times the father was observed carrying the infant over seven days by the number of total observations recorded for that infant during that interval. Similarly, we calculated mother carry by dividing the number of times the mother was observed carrying the infant by the number of total observations recorded for that seven-day interval. Parent carry was calculated by adding the times either the mother and father were observed carrying the infant over a seven-day period divided by the total number of observations for that sampling period. These calculations gave us weekly scores of the proportion of time the infant was carried by the father, the mother, or either parent, with possible values ranging from 0 to 1. Data for this study are available via Open Access (http://doi.org/10.5281/zenodo.4480404).

Data Analyses

We first wanted to examine change in parent social affiliation over a 16-month period. In R Statistical Software (version 4.0.3, R Core Development Team, 2020), we performed a Shapiro Wilk test of normality (Royston, 1983), removed two extreme outliers, and used Levene's test using the car package (Fox & Weisberg, 2019) to test for homogeneity of variance (Schultz, 1985). Using the lme4 package (Bates, Mächler, Bolker, & Walker, 2015), we used a general linear mixed model (LMM) to determine whether period of time (fixed effect) predicted the proportion of time a pair spent in affiliative contact. We included pair ID as a random, repeated-measures effect. We performed a log likelihood ratio test to compare the fit of our model to that of a null model. To compare the mean values of affiliation between time points, we conducted a Tukey's Test post-hoc analysis, correcting for multiple comparisons, using the multcomp package (Hothorn, Bretz, & Westfall, 2008). Tests were two-tailed and the significance threshold was set at .05.

We next wanted to determine how parenting behaviors impacted the proportion of time parents spend in social affiliation with each other during the first eight months of an infant's life. Upon visual inspection of the data, it appeared affiliation followed a quadratic trend (Figure 1). To empirically test which trajectory best explained our data, different growth models that included no growth, linear growth, and quadratic growth were applied to the data using SAS 9.4 using PROC NLMIXED (SAS Institute, Cary, NC). These tests were run prior to including model covariates, but determined which growth model we would use to test for the effects of covariates. This model is subject-specific, allowing for a general growth model to characterize the population, but also allowing the coefficients of the growth model to be unique to the individual dyad. Based on a comparison of AIC values, the quadratic growth model best fit our data.

We then compared measures of fit such as log likelihood and the information criterion of Akaike (AIC; Akaike, 1974) to determine whether adding random effects for the intercept, trough value of affiliation (value at which the inflection point occurs in the quadratic trajectory when affiliation begins to increase again), and time to trough value of affiliation. Deviance tests indicated that a quadratic growth model that included a random effect for each of the growth coefficients provided the best relative fit.

We then built on our baseline model by adding time-varying and fixed coefficients. Our time-varying covariates were the proportion of time the father spent carrying the infant each week, proportion of time the mother spent carrying the infant, and the proportion of time either parent was observed carrying the infant during each seven-day interval. We used pair-centering and grand-mean-centering to determine the effect of each time-varying covariate within a pair, controlling for all other covariates, and between pairs, including the effects of the other predictors. The fixed covariates were the length of time a pair had been together at the time the infant was born (pair tenure) and the age at which the infant became independent (defined as the point at which the infant has been observed “off” for ten consecutive observations). Fixed covariates were centered about their respective means. We chose to examine the effects of pair tenure on affiliation because a previous study showed that well-established pairs were more likely to be observed tail-twining during scan samples than newly-formed pairs (Rothwell et al, 2020). We chose to examine infant-specific predictors because the presence of an infant predicts a decrease in proportion of time pairs spend in affiliative contact during the first year that a pair is together (Witczak, Blozis, & Bales, *in prep*).

Given that a quadratic growth model provided the best relative fit to the social response scores, a version of the model with interpretable parameters was applied to test the effects of covariates (Cudeck & du Toit, 2002). Letting y_{ij} denote the social response for titi monkey pair *i* at week *j*, the quadratic growth model was parameterized as

$$y_{ij} = \beta_{yi} - (\beta_{yi} - \beta_{0i})\left(\frac{week_{ij-1}}{\beta_{xi}} - 1\right)^2 + \varepsilon_{ij} \qquad (1)$$

where, for titi monkey pair *i*, β_{0i} represents the response at week 1, β_{yi} represents the trough response, and β_{xi} represents the week at which the trough response occurs. In the model, each of the coefficients is a sum of a fixed effect that relates to the population and a random effect that relates to titi monkey pair *i*. For example, $\beta_{0i} = \beta_0 + u_{0i}$, where β_0 denotes the expected response for the population at week 1 and u_{0i} is the random effect for titi monkey pair *i*. Positive values of u_{0i} for an animal pair would indicate that a titi monkey pair’s response at week 1 is higher than the expected value of the population, and a negative value indicate that an animal pair's response at week 1 is lower than the expected value of the population. Finally, the residual of the model is denoted by ε_{ij}.

Under the quadratic growth model in Equation (1) the residual was assumed to be normally distributed with a mean of zero and variance σ_{ε}^{2}. The variance was assumed to be homogeneous across animal pairs. Specifically, the residual variance $\sigma_{\varepsilon i}^{2}$ was assumed to follow a lognormal model (c.f.: Hedeker et al., 2008):

$$\sigma_{\varepsilon i}^{2} = \exp(\tau_0) \tag{2}$$

where τ_0, when exponentiated, is the (geometric) residual variance for a titi monkey pair.

The three quadratic growth coefficients (β_{0i}, β_{yi}, and β_{xi}) were then predicted by the covariates, with each covariate centered about their respective sample mean. First, the effects of covariates were evaluated individually. In evaluating the effects of the length of time a pair had been together, for instance, each of the growth coefficients was regressed as follows:

$$\beta_{0i} = \beta_{00} + \beta_{01} PairTenure_i + u_{0i}$$

$$\beta_{yi} = \beta_{y0} + \beta_{y1} PairTenure_i + u_{yi}$$

$$\beta_{xi} = \beta_{x0} + \beta_{x1} PairTenure_i + u_{xi}$$

where β_{00}, β_{y0}, and β_{x0} represent the expected response at week 1, the trough response, and the week at which the trough response occurs, respectively, for titi monkey pairs whose pair tenure score was at the sample mean. The coefficients β_{01}, β_{y1}, and β_{x1} represent the expected unit change in each of the coefficients for a one-unit increase in $PairTenure_i$. The residuals of the three equations, u_{0i}, u_{yi}, and u_{xi}, denote the random effects conditional on $PairTenure_i$. In each of the models fit to the data, each of the three random growth coefficients could covary.

Next, the full set of covariates were included in a larger model to provide tests of the covariates with the effects being statistically adjusted for other model covariates. Effects that were not statistically significant at the .05 level when tested independently were not included in the larger model and a final, relatively parsimonious version of the model was used for interpretation. It is important to note that the significance of fixed and random effects was only interpreted in this one final model; therefore, post-hoc corrections were not needed for this final model.

Results

We first examined general changes in social affiliation between titi monkey pair mates over the eight months prior to infant birth and the eight months following infant birth. We collected an average of 1030.36 (SE = 71.66) scan samples per pair over this period of time (range = 117 – 1497). Analyses were based on a total of 22,668 observations. Initially, our data were not normally distributed (W = 0.93, $p < .001$); however, when we plotted our data, we identified two outliers (Figure 1). The same pair spent 80.9% and 79.0% of their observations in affiliative contact during the first period (8 to 5 months pre-birth of the infant) and second period (4 to 0 months pre-birth of the infant), respectively. When we removed those two outliers, our data were normally distributed across each period (Table 2; Figure 2). Levene's test also indicated equal variances (F (3, 82) = 0.82, p = .49). Because our data were normally distributed and did not violate assumptions of homogeneity of variance, we fit a general linear mixed-effects model to our data. The period of time had a significant effect on proportion of time in affiliative contact (X2(3) = 67.23, $p < .0001$. Tukey's test revealed significant differences between nearly all four-month periods of time (Table 3). Affiliation at T2 (4 to 0 months pre-birth of infant; M = 0.39, SE = 0.02) was higher than affiliation at T1 (8 to 5 months pre-birth of infant; M = 0.32, SE = 0.02, $p < .001$), T3 (4 to 0 months pre-birth of infant; M = 0.31, SE = 0.02, $p < .001$) and

T4 (5 to 8 months post-birth of infant; M = 0.21, SE = 0.02, p < .001). Pair affiliation was also lower at T4 than it was at T1 (p < .001; Table 3) and T3 (p < .001).

We next wanted to determine why the proportion of time pairs spend in affiliative contact decreased after the birth of the infant. Data from the first eight months post-birth of the infant were used for analyses, and time was binned into seven-day periods to capture the effects of rapid changes in infant independence. We collected an average of 592.67 (SE = 54.73) observations per pair (range = 168–1429), and analyses were based on a total of 12,374 observations. A Shapiro-Wilk test revealed that data were normally distributed for 26 of the 35 seven-day periods (Table 4; Figure 3), so we decided not to remove any outliers or transform our variables. Levene's test also suggested our data did not violate assumptions of homogeneity of variance (F (34, 641) = 0.83, p = .74). Therefore, we were able to proceed with our nonlinear mixed-effects modeling.

Given the pattern of affiliation over the eight-month period post-infant birth (Figure 3), we first determined whether a no growth, linear growth, or quadratic growth model best fit our data. Deviance tests indicated a quadratic growth model provided best relative fit (Table 5). Additionally, as we fit our no growth, linear growth, and quadratic growth models, residual variance decreased from 0.36, to 0.11, to 0.07, respectively. Therefore, the quadratic model explained the most variance in the data. These findings suggest affiliation declines after the birth of an infant, but then hits an inflection point and begins to rise again after a period of time. We added random effects one by one to determine whether dyads were quantitatively different in their starting levels of affiliation, their trough levels of affiliation, and the time when they reached their trough level of affiliation. Based on a comparison of model fit, the model with all three random effects best fit our data (Table 6) and resulted in the lowest residual variance ($\sigma_{\varepsilon i}^2 = 0.05$).

We then evaluated the effects of each covariate independently. Our covariates were the length of time a pair had been together (pair tenure), the time at which an infant was considered independent (independent), the proportion of time the father spent carrying the infant (father carry), the proportion of time the mother spent carrying the infant (mother carry), and the proportion of time either parent spent carrying the infant (parent carry). Covariates were entered at all three levels (intercept, trough, and time to trough). Covariates that were statistically significant were added to the final, full model (Table 7). Because including all three measures of father carry, mother carry, and parent carry in our final full model would violate assumptions of independence, we chose to examine residual variance to determine which parameter to include in our final model. Compared to models with the other carrying-related covariates, the model that included father carry as a covariate resulted in the lowest residual variance ($\sigma_{\varepsilon i}^2 = 0.02$; Table 7). Upon examining the data, father carry also varied more at each time point (Figure 4) than mother carry (Figure 5) and parent carry (Figure 6). For these two reasons, we decided to include the father carry covariates (grand-mean centered and pair-centered) in our final model and did not include the mother carry or parent carry covariates.

Our final full model included the effects of father carry (grand-mean centered and pair-centered) on the intercept, the effects of infant age of independence and pair tenure on the trough value, and the effects of infant age of independence, pair tenure, and father carry (grand-mean centered and pair-centered) on the time to trough response (Table 8). The population mean proportion of time spent in affiliative contact when infants are born is 0.40 (SE = 0.21), the population mean affiliation score at the trough (or inflection point) is 0.17 (SE = 0.11), and the population mean time to trough is approximately 32.60 weeks (SE = 5.80 weeks). Between pairs, including the effects of the other covariates in the model, fathers who spend more time carrying their infant than the mean time fathers carry infants have lower initial levels of time spent in affiliative contact (β_{01a} = -2.21, SE = 0.86, p = .02). This effect size is medium (D = -0.55, Hedges, 1982). If an infant becomes independent at an age that is later than the mean time infants become independent, then the proportion of time parents spend in affiliative contact at the trough (or inflection point) is higher (β_{y1} = 3.94, SE = 1.59, p = .02, D = 0.53). These findings suggest that these pairs would not drop as low in affiliation as pairs whose infants become independent earlier. Pairs who have been together for longer than the mean pair tenure have a lower trough value than the mean population (β_{y2} = -0.38, SE = 0.12, p = .004, D = -0.69). The results of this model suggest these pairs who have been together longer would be expected to drop lower than 17.1% time in affiliative contact at their trough. Between pairs, including the effects of the other covariates in this model, fathers who spend more time carrying the infant than the mean time fathers carry infants hit their trough sooner (β_{x3a} = -3.46, SE = 1.49, p = .03, D = -0.50). While this effect is small, this suggests that pairs with fathers who spend a lot of time carrying infants will switch to an increase in affiliation sooner than pairs with fathers who generally don't carry infants as much when we account for the other covariates in the model. Interestingly, within a pair, when we control for the effects of the other covariates, pairs with fathers who spend more time carrying their infants reach that time to trough later (β_{x3b} = 1.09, SE = 0.51, p = .05, D = 0.46). These findings suggest that, excluding the effects of the other covariates, pairs with fathers who spend more time carrying their infant than the population mean take a longer time to hit that inflection point where affiliation begins to rise again.

All of our random effects were significant with the exception of the trough value, suggesting pairs may not differ significantly in that trough value of social affiliation (φ_y = 0.11, SE = 0.16, p = .49, D = 0.15). Pairs did significantly vary in their initial levels of affiliation (φ_0 = 1.27, SE = 0.26, p < .001, D = 0.98) and their time to trough affiliation value (φ_x = 0.84, SE = 0.17, p < .001, D = 1.09). Individual levels of affiliation and trough values are positively correlated ($\rho_{0,y}$ = 1.00, SE = 0.01, p < .001, D = 36.88), suggesting pairs who are more affiliative at the beginning of the sampling period have a higher trough value than those who are less affiliative initially. Initial levels of affiliation and the time to trough are also positively correlated ($\rho_{0,x}$ = 0.78, SE = 0.33, p = .03, D = 0.50), meaning if a pair starts off higher in affiliation, then it will reach that inflection point later and therefore take a longer time to begin the increase in affiliation. The trough value and the time to trough were also positively correlated ($\rho_{x,y}$ = 0.81, SE = 0.33, p = .02, D = 0.53), meaning those with a higher trough value also take longer to reach that trough point at which they would make the switch to increasing in affiliation.

Our final full model also had lower residual variance than the models without covariates and any of the models that only included one covariate ($\sigma_{\varepsilon i}^2$ = 0.02). Deviance tests also indicated that this model fit better than our quadratic model that included all three random effects but did not include any covariates ($X^2(8)$ = 39.2, p < .001). AIC was also smaller for this full model (AIC = 2135.0) than it was for the quadratic model with three random effects and zero additional covariates (AIC = 2194.3). Therefore, compared to all other models tested, this final full model best explained our data.

Discussion

Our exploration of intra-pair affiliation across the 16 months centered on the birth of a pair's first infant revealed important changes in pair affiliation associated with this major life history event. First, our analyses revealed temporal differences in pair affiliation across time. Affiliation between pair mates was highest in the second time period, during pregnancy 4 to 0 months prior to the birth of the infant. There are important potential biological reasons for this. It is possible that there is something unique about pregnancy, such as decreased mobility for the female that supports increased affiliation within pairs during this period. Within titi monkey pairs, females control proximity between pair mates—both in terms of approaching and withdrawing (Dolotovskaya, Walker, et al., 2020). With restricted mobility and lower energy during pregnancy, females may withdraw less often simply by virtue of being more sedentary, leading to higher calculations of affiliation using our sampling method. It is also possible that lower levels of affiliation at other time points drives the pattern of pair affiliation. T2 coincides not only with pregnancy, but also with longer relationship tenure relative to T1. Titi monkey pairs may simply increase in affiliation across pair tenure. There is limited data on intra-pair affiliation across time, but Rothwell et al., (2020) found that well-established pairs were more likely to be observed tail-twining during scan samples than newly-formed pairs, suggesting that at least this measure of affiliation may increase with pair tenure. Titi monkey rates of affiliative behaviors (e.g., grooming, proximity) are low at the time of pairing and increase dramatically during the first week, at which point they become stable across the next month (Hoffman, 1998). Other studies have not examined average levels of affiliation between pair mates across time in this way and indeed it would be difficult to examine such behavior without including the influence of infant presence as titis with unrestricted reproduction tend to give birth within the first year of pairing (Valeggia et al., 1999).

We expected to see reductions in pair affiliation following birth (T3 and T4) as a consequence of energetic re-allocation toward infant rearing. Our findings align with previous research in titi monkeys which found reductions in social behavior and affiliation (Dolotovskaya & Heymann, 2020; Dolotovskaya, Walker, et al., 2020) and humans, which found reductions in relationship quality (Richter, Krämer, Tang, Montgomery-Downs, & Lemola, 2019) and relationship functioning (Doss, 2009) following the birth of an infant. While we expected lower rates of affiliation postpartum relative to prepartum, we did not expect lower rates of affiliation in T4 compared to T3. Our model estimated an average time to trough of 32.6 weeks (approximately 228 days), which demonstrates a longer amount of time of decreasing affiliation than we expected. We predicted that affiliation between parents would begin to increase close to the time when infants transition to behavioral independence, which was approximately 150 days for our sample. Given these results, it is unclear when and whether intra-pair affiliation fully rebounds after partners become parents, especially given that titi monkeys reproduce annually (Valeggia et al., 1999). Future research should expand the postnatal window of interest to include the birth of a subsequent infant in order to better understand how affiliation changes across this interim.

In order to examine how pair affiliation changes in response to the demands of infant care, we used nonlinear mixed effects modeling, specifying a quadratic trajectory, and included the time-varying fixed effect of paternal carry as well as the fixed effects of pair tenure and age of infant independence. Our model also included three random effects, allowing for estimates of initial levels of affiliation, trough values of affiliation, and time to trough values of affiliation to be independently estimated across pairs. The model identified several effects. As predicted, affiliation between pair mates decreased following the birth of an infant, but initial rate of affiliation (intercept), length of the decline in affiliation (time to trough), and depth of the decline in affiliation (trough) differ with our covariates. During the first week following their infant's birth, new parents spent an average of 40.4% of their time in affiliative proximity or contact with their pair mate, but over the course of an average of 32.6 weeks, this rate of affiliation declined to an average of 17.1%. There was significant variation between pairs in initial rate of affiliation and this intercept covaried with both the time to, and value of, the trough. Pairs with a high rate of initial affiliation also had higher minimum rates of affiliation and arrived at their lowest rates later. In other words, pairs that spent more time in affiliative contact during the first week after their infant was born had a less steep decline in affiliation across the infant's development, but these pairs also took longer to arrive at this minimum value and switch from decreasing to increasing in affiliation. There was no significant variation between pairs in the minimum proportion of time spent in affiliative contact, but there was significant variation in the time it took for pairs to reach their minimum rate of affiliation and transition from decreasing to increasing in affiliation. Time to this nadir was positively correlated with the minimum value such that pairs with higher minimum values took longer to reach that value (i.e., they had a longer, slower decline in affiliation).

Pairs in which the father carried the infant more often than the mean had lower rates of pair affiliation during the first postpartum week. Pairs in which the father provides a higher amount of care may shift their energetic resources away from pair-focused activities toward infant-focused activities early on. Dolotovskaya & Heymann (2020) found that female wild coppery titi monkeys prioritized eating over rest and some social activities (likely to support the metabolic price of lactation), while males prioritized resting over eating. Perhaps in pairs in which the father provides more paternal care, the female is free to engage in foraging and feeding activities early on and it is her reprioritization of her time budget that leads to decreased time spent in affiliative contact with her mate. Furthermore, female titi monkeys act as the primary initiator of social proximity and contact (Dolotovskaya, Walker, et al., 2020) and will avoid being in proximity to their mate if he is carrying the infant (Reeder, 2001). Given this information in conjunction with our data, females may avoid affiliating with their partner if he is carrying the infant in an effort to prioritize feeding and/or avoid proximity with the infant—an effect which would be exacerbated by high levels of male infant carrying. In turn, titi monkey infants have a preferential attachment to their fathers and actively solicit moving from their mother to their father following the end of a nursing bout (Mendoza & Mason, 1986). This highlights to potential role of the infant's attachment to the father as a potential impetus for changes in affiliation and relationship maintenance between new parents.

A within-pairs comparison revealed that pairs in which the father carried the infant more often than the mean hit the nadir of affiliation later. While this measure excludes the effects of our other covariates (i.e., pair tenure and infant age at independence), it does signal that paternal infant carrying takes a toll on the time a pair spends in affiliative contact. When examining between-pairs effects, we found the opposite effect: pairs in which the father carried the infant more often than the mean switch to an increase in affiliation sooner, signaling that these pairs may recover their rate of affiliation faster than pairs in which the father carries the infant less than the mean. As only the male and female are available to carry the infant, pairs in which the male carries the infant less often are also pairs in which the female carries the infant more often. Perhaps a high rate of maternal carrying in addition to supporting the infant through lactation results in the pair taking longer to increase their affiliation as the female further prioritizes eating to sustain this heavy energetic load.

Finally, infant independence and pair tenure affected minimum rates of affiliation. Pairs whose infants became independent later than the mean spent more time in affiliative contact at the nadir. While studies in several primate species support the theory put forth by Altmann (1980) that new mothers direct energy away from social activities in favor of eating, they also propose more nuance. Among black howler monkeys (Dias et al., 2011) and coppery titi monkeys (Dolotovskaya & Heymann, 2020), females maintain time allocated to social activities (e.g., grooming) for as long as possible, preferring to reduce resting time prior to reducing social time. Perhaps having an infant that is dependent on its parents for longer impacts the way in which the mother allocates her time budget such that more social activity is preserved. Another explanation may be that fathers promote infant independence in order to obtain greater social access to their partner. Thus, mothers who are more tolerant of being in proximity to their partner while he carries the infant may have infants who are carried longer because the father has less cause to hasten their independence. In reference to pair tenure, pairs that had been together longer than the mean had lower minimum levels of affiliation. Perhaps pairs that have been together longer do not need to employ these behaviors, or are employing different behaviors, in order to maintain their pair bond.

Broadly, this work supports previous findings in both titi monkeys and humans and continues the narrative that the birth of offspring initiates a decline in relationship maintenance behaviors such as intra-pair affiliation. But the implication that relationship partners allocate their energy away from relationship maintenance to prioritize infant care may over-simplify this phenomenon. The impact of paternal care, length of relationship, and infant independence all play various roles in shaping the timing and degree of the decline in affiliation and though our research illustrates the pattern of change across time, the underlying mechanism behind such change remains theoretical.

Limitations

There were some limitations of the current work. First, our measure of pair mate behavior was conditional, meaning that both animals needed to participate in order for the behavior to be scored. Since the measure is not individual-specific, we cannot know which animal initiated or refused affiliative contact and steered these interactions. Second, some pairs in our sample did not have a complete dataset for T1 because they became pregnant less than four months after being paired. Due to our small sample size, it is possible that the exclusion of these pairs from T1 biased the population mean of affiliation at that time point.

Future directions

Overall, these analyses tell us that caring for an infant is costly—and one of the things it costs new parents is time spent together, specifically time spent maintaining their pair bond. This decline may be due to active avoidance by one parent when the partner is carrying the infant or simply a casualty of prioritizing other activities (e.g., eating). Given the length of time in which affiliation continues to decline, further study is needed to identify what factors co-occur with the nadir in affiliation and spur increases in affiliation rates. One mechanism that may contribute to the length of time in which affiliation declines is parental sleep. In humans (Bayer, Hiscock, Hampton, & Wake, 2007; Lee, Zaffke, & McEnany, 2000; Yamazaki, Lee, Kennedy, & Weiss, 2005) and nonhuman primates (Fite et al., 2003), parental sleep declines following the birth of an infant. Decreased sleep is associated with impaired affective processing, namely difficulty in perspective-taking (Shapiro, Gottman, & Carrère, 2000), perceiving events to have a negative bias (Tempesta et al., 2010), and an increased tendency to assign blame (Kahn-Greene, Lipizzi, Conrad, Kamimori, & Killgore, 2006). It is little wonder then, that new parents experience more negative communication and higher problem intensity as well as decreased conflict management (Doss, 2009). Postpartum decrease in sleep is typically more severe for mothers compared to fathers and can persist even after the infant gains nutritional independence (Fite et al., 2003; Richter et al., 2019). Parental sleep has not yet been investigated in titi monkeys, but exploration of the timing of postpartum sleep recovery could provide insight into the recovery of affiliative behavior between pair mates.

Future research should also investigate how other behaviors implicated in pair-bond maintenance are affected by the birth of an infant, going beyond affiliation to include other species-typical behaviors. For instance, vocal duetting is a hallmark behavior of titi monkeys (Robinson, 1979) and has been shown to convey information about individual identity (Lau et al., 2020), pair tenure (Clink, Lau, & Bales, 2019), age (Clink et al., 2019), and kinship (Clink et al., *in review*). Given the complexity of these territorial duet vocalizations (Robinson, 1981) and their importance in daily titi life (Mason, 1966), titis may alter aspects of their duets with changes in parental status.

Given the duration during which pair affiliation decreased following the infant's birth, dyads may become pregnant prior to regaining prepartum rates of affiliation. Future research should seek to understand whether recovery occurs, what effect the birth of a second infant has on intra-pair affiliation, and whether there is a cumulative effect of subsequent births on adult affiliation.

Finally, the next chapter to this work should focus directly on the impact of caregiving experience (i.e., paternal care, maternal care) on the pairing and parenting success of these infants in adulthood. Tracing multiple generations of parenting behavior and pair affiliation would allow us to form a more complete picture of how titi monkeys become the social animals they are and form the attachment network that is the hallmark of their species. Compelling research in humans demonstrates that early social interactions between infant and caregiver shape cross-generational transmission of social affiliation (Feldman, Gordon, & Zagoory-Sharon, 2010). While the implications of this cross-generational transmission of social affiliation has been studied in connection with infants' social behavior, it would be interesting to assess these infants' performance in future pair bonds, specifically in regards to pair bond maintenance behaviors.

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Tables

Table 1. Ethogram for social behaviors.

Behavior	**Definition**
Affiliative Social Interactions	
Passive Contact	Male’s and female’s bodies are in physical contact that does not include tail-twining.
Social Proximity	Animal’s body (excluding the tail) is within arm’s length (approximately 6 inches) of another animal (excluding the tail).
Tail Twine	Male and female tails are intertwined for at least one full turn.
None	Male and female are not in passive contact, social proximity, or tail twining.
Infant Carry Interactions	
Father Carry	Infant is being carried by the father. Needs to have both back feet on the father to count as being carried (can have hands off father but as long as both feet are on father will count as a carry).
Mother Carry	Infant is being carried by the mother. Needs to have both back feet on the mother to count as being carried (can have hands off mother but as long as both feet are on father will count as a carry).
Off	Infant is not being carried by mother or father. If hands are touching a parent but feet are off of the parent this would be scored as “off”.

Table 2. Proportion of time pairs spend in affiliative contact across the 4 four-month periods. Mean and standard error of the mean (SE) are reported with results from the Shapiro-Wilk test (W and p value), as well as measures of skewness and kurtosis.

Variable	**Mean**	**SE**	**W**	**p**	**skewness**	**kurtosis**
T1 (8-5 months pre-infant birth)	0.32	0.02	0.98	.84	-0.09	-0.96
T2 (4-0 months pre-infant birth)	0.39	0.02	0.98	.92	0.12	-0.82
T3 (0-4 months post-infant birth)	0.31	0.02	0.96	.43	0.35	-0.97
T4 (5-8 months post-infant birth)	0.21	0.02	0.94	.21	0.54	-0.49

Table 3. Results from post hoc analyses using Tukey's post-hoc test. Period 1 = 8 to 5 months pre-birth of infant; Period 2 = 4 to 0 months pre-birth of infant; Period 3 = 0 to 4 months post-birth of infant; Period 4 = 5 to 8 months post-birth of infant.

Comparison	**Estimate**	**SE**	**p**
T2-T1	0.07	0.02	< .001
T3-T1	-0.02	0.02	.68
T4-T1	-0.12	0.02	< .001
T3-T2	-0.09	0.02	< .001
T4-T2	-0.18	0.02	< .001
T4-T3	-0.1	0.02	< .001

Table 4. Proportion of time pairs spend in affiliative contact across the 35 seven-day periods. Mean and standard error of the mean (SE) are reported with results from the Shapiro-Wilk test (W and p value), as well as measures of skewness and kurtosis.

Week	**Mean**	**SE**	**W**	**p**	**skewness**	**kurtosis**
1	0.43	0.03	0.93	.23	0.28	-1.33
2	0.41	0.02	0.96	.61	-0.35	0.92
3	0.34	0.04	0.89	.03	0.49	-1.36
4	0.32	0.04	0.95	.39	0.30	-1.06
5	0.37	0.04	0.95	.35	0.42	-0.95
6	0.33	0.03	0.85	.01	1.25	0.78
7	0.31	0.03	0.90	.05	0.43	-1.36
8	0.31	0.03	0.96	.58	0.00	0.99
9	0.28	0.03	0.92	.07	0.29	-1.37
10	0.26	0.03	0.97	.75	0.11	-1.08
11	0.25	0.02	0.98	.92	-0.03	0.35
12	0.26	0.03	0.97	.66	0.44	-0.51
13	0.26	0.03	0.98	.84	-0.03	-0.95
14	0.23	0.02	0.96	.48	0.38	1.02
15	0.25	0.03	0.95	.34	0.44	-0.70

16	0.27	0.04	0.96	.46	0.53	-0.48
17	0.23	0.03	0.91	.06	-0.07	-1.58
18	0.24	0.03	0.95	.36	0.33	-1.11
19	0.21	0.03	0.91	.07	1.11	1.71
20	0.21	0.02	0.97	.83	-0.21	-0.37
21	0.16	0.03	0.92	.12	0.41	-0.86
22	0.22	0.03	0.86	.01	1.17	0.39
23	0.23	0.05	0.65	.00	2.56	6.72
24	0.16	0.03	0.95	.34	0.38	-1.01
25	0.19	0.02	0.83	.00	1.18	0.31
26	0.23	0.03	0.94	.22	0.02	-1.15
27	0.19	0.03	0.94	.33	0.51	-0.55
28	0.22	0.03	0.90	.08	0.74	-0.74
29	0.21	0.04	0.89	.04	0.78	-0.25
30	0.24	0.03	0.96	.62	-0.03	-1.31
31	0.21	0.04	0.88	.02	1.20	0.98
32	0.25	0.04	0.97	.74	0.37	-0.70
33	0.26	0.03	0.86	.01	1.43	2.17

34	0.22	0.03	0.98	.92	0.39	-0.29
35	0.20	0.04	0.90	.10	0.76	0.45

Table 5. Indices of model fit, where q is the total number of model parameters. -2lnL is -2 times the log likelihood. AIC is the Akaike information criterion. Smaller values of the AIC indicate better fitting models. X^2(df) is a deviance statistic for model fit comparisons.

Model	**q**	**-2lnL**	**AIC**	**Models compared**	**X^2(df)**	**p**
No growth	3	2371.2	2377.2			
Linear	6	2228.3	2240.3	No growth vs linear	142.9(3)	<.001
Quadratic	10	2182.2	2202.2	Linear vs quadratic	46.1(4)	<.001

Table 6. Indices of model fit, where q is the total number of model parameters. -2lnL is -2 times the log likelihood. AIC is the Akaike information criterion. Smaller values of the AIC indicate better fitting models. (*df*) is a deviance statistic for model fit comparisons.

Model	**q**	**-2lnL**	**AIC**	**Models compared**	**X^2(df)**	**p**
intercept	11	2244.3	2254.3			
intercept + trough	12	2196.7	2210.7	intercept vs intercept + trough	47.6(1)	<.001
intercept + trough + time to trough	13	2174.3	2194.3	intercept + trough vs intercept + trough + time to trough	22.4(1)	<.001

Table 7. Individual covariate effects on the response at week 1, trough response, and time of trough response. gmc = grand-mean-centered; pc = pair-centered; * = $p < .05$.

Covariate	β_{01}	β_{y1}	β_{x1}	$\sigma_{\varepsilon i}^2$
Pair Tenure	0.120	-0.421*	-0.605*	0.048
Independence	7.582	4.685*	8.577*	0.043
Father Carry (gmc)	-0.649	-0.663	-2.364*	0.024
Father Carry (pc)	-0.922*	0.165	1.858*	0.024
Mother Carry (gmc)	-1.859	1.894	3.688	0.043
Mother Carry (pc)	0.648	-0.108	-1.049*	0.043
Parent Carry (gmc)	0.192	-0.003	-4.095*	0.025
Parent Carry (pc)	0.762	-0.388	2.445*	0.025

Table 8. Parameter estimates from final full model for the effects of each covariate on the proportion of time pairs spent in affiliative contact at week 1 (intercept), trough affiliation score, and time of trough response. gmc = grand-mean-centered; pc = pair-centered; D = Cohen's D (effect size)

Parameter		**Estimate**	**SE**	**DF**	**t Value**	**Pr > |t|**	**95% Confidence Limits**		**D**
Fixed Effects									
Mean intercept	$\boldsymbol{\beta_{00}}$	4.04	0.21	19	19.59	<.0001	3.61	4.47	4.18
Mean trough	$\boldsymbol{\beta_{y0}}$	1.71	0.11	19	15.78	<.0001	1.48	1.94	3.36
Mean time to trough	$\boldsymbol{\beta_{x0}}$	3.26	0.58	19	5.61	<.0001	2.04	4.48	1.20
Father carry (gmc; intercept)	$\boldsymbol{\beta_{01a}}$	-2.21	0.85	19	-2.59	.02	-4.00	-0.42	-0.55
Father carry (pc; intercept)	$\boldsymbol{\beta_{01b}}$	-0.58	0.54	19	-1.08	.29	-1.72	0.55	-0.23
Infant age of independence (trough)	$\boldsymbol{\beta_{y1}}$	3.94	1.59	19	2.48	.02	0.62	7.26	0.53
Pair Tenure (trough)	$\boldsymbol{\beta_{y2}}$	-0.38	0.12	19	-3.23	.00	-0.63	-0.13	-0.69
Infant age of independence (time of trough)	$\boldsymbol{\beta_{x1}}$	5.71	5.31	19	1.08	.30	-5.40	16.81	0.23
Pair Tenure (time of trough)	$\boldsymbol{\beta_{x2}}$	-0.24	0.13	19	-1.80	.09	-0.52	0.04	-0.38
Father carry (gmc; time of trough)	$\boldsymbol{\beta_{x3a}}$	-3.46	1.49	19	-2.33	.03	-6.57	-0.35	-0.50
Father carry (pc; time of trough)	$\boldsymbol{\beta_{x3b}}$	1.09	0.51	19	2.14	.05	0.02	2.16	0.46
Random Effects									
residual variance	$\boldsymbol{\tau_0}$	0.15	0.06	19	2.64	.02	0.03	0.27	0.56
individual (intercept)	$\boldsymbol{\varphi_0}$	1.27	0.27	19	4.61	<0.001	0.69	1.84	0.98
	$\boldsymbol{\rho_{0,y}}$	1.00	0.01	19	172.96	<.0001	0.98	1.01	36.88
individual (intercept, trough)									
individual (trough)	$\boldsymbol{\varphi_y}$	0.11	0.16	19	0.70	.49	-0.22	0.44	0.15
	$\boldsymbol{\rho_{0,x}}$	0.78	0.33	19	2.36	.03	0.09	1.46	0.50
individual (intercept, time of trough)									
	$\boldsymbol{\rho_{x,y}}$	0.81	0.33	19	2.49	.02	0.13	1.49	0.53
individual (trough, time of trough)									
individual (time of trough)	$\boldsymbol{\varphi_x}$	0.84	0.17	19	5.09	<.0001	0.49	1.18	1.08

Figure Legends

Figure 1. Proportion of time pairs spend in affiliative contact over 4 four-month periods. Outliers included in the figure. Each point is an observed score for an individual pair at each time point. Boxplots represent median value of affiliative contact with lower and upper hinges corresponding to the first and third quartiles. A violin plot overlays the data for each time period, illustrating the distribution of the variables.

Figure 2. Proportion of time pairs spend in affiliative contact over 4 four-month periods. Outliers were excluded in figure. Each point is an observed score for an individual pair at each time point. Boxplots represent median value of affiliative contact with lower and upper hinges corresponding to the first and third quartiles. A violin plot overlays the data for each time period, illustrating the distribution of the variables.

Figure 3. Proportion of time pairs spend in affiliative contact over 35 seven-day periods. Each point is an observed score for an individual pair at each time point. Boxplots represent median value of affiliative contact with lower and upper hinges corresponding to the first and third quartiles. A violin plot overlays the data for each time period, illustrating the distribution of the variables.

Figure 4. Proportion of time fathers spend carrying infants over 35 seven-day periods. Each point is an observed score for an individual pair at each time point. Boxplots represent median value of affiliative contact with lower and upper hinges corresponding to the first and third quartiles. A violin plot overlays the data for each time period, illustrating the distribution of the variables.

Figure 5. Proportion of time mothers spend carrying infants over 35 seven-day periods. Each point is an observed score for an individual pair at each time point. Boxplots represent median value of affiliative contact with lower and upper hinges corresponding to the first and third quartiles. A violin plot overlays the data for each time period, illustrating the distribution of the variables.

Figure 6. Proportion of time parents spend carrying infants over 35 seven-day periods. Each point is an observed score for an individual pair at each time point. Boxplots represent median value of affiliative contact with lower and upper hinges corresponding to the first and third quartiles. A violin plot overlays the data for each time period, illustrating the distribution of the variables.

Figure 1

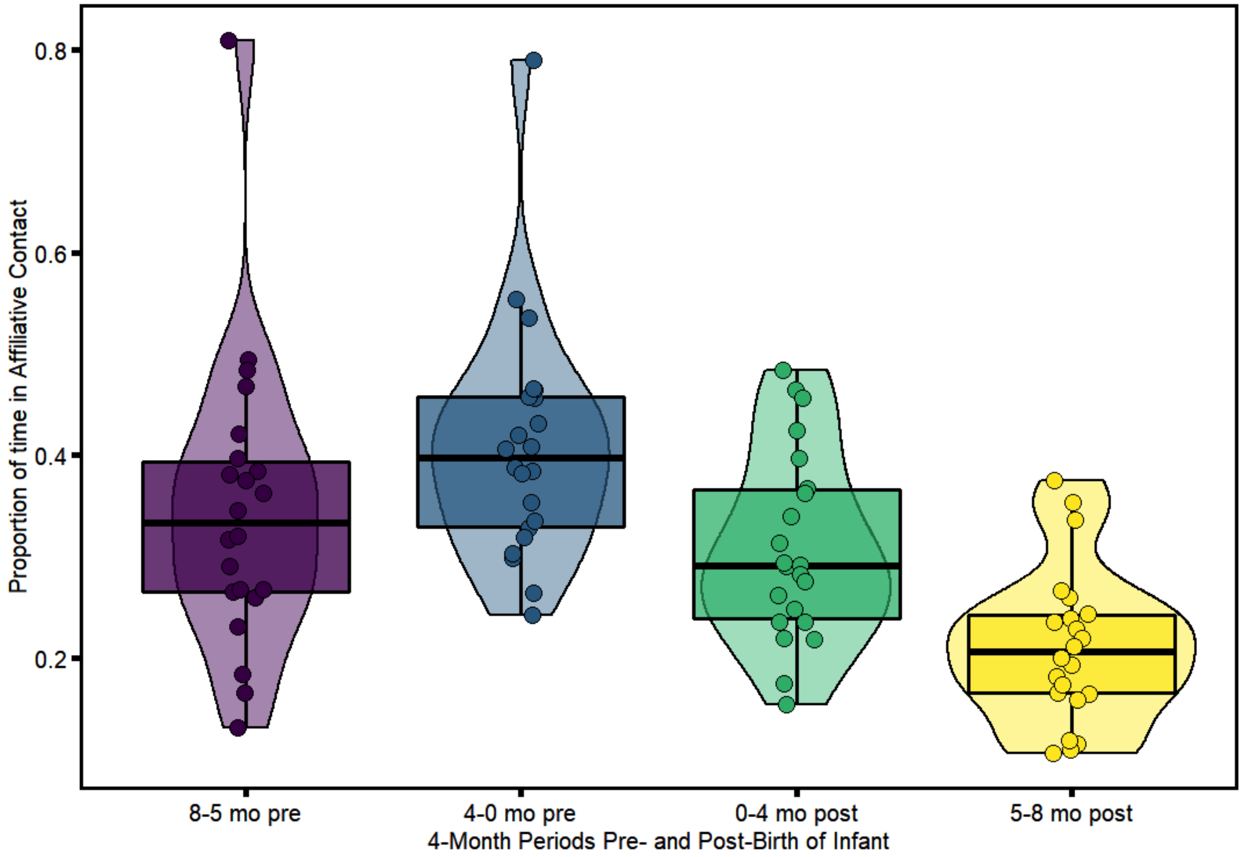
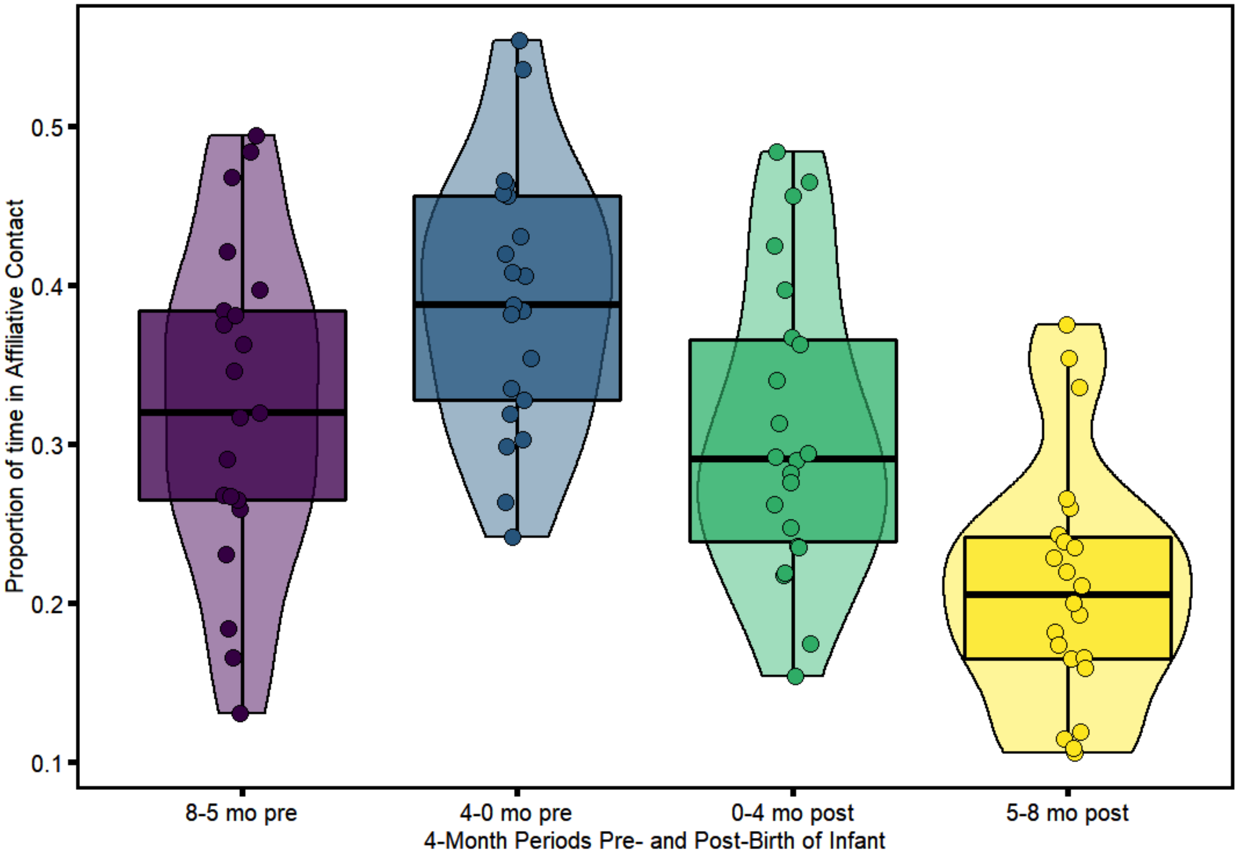
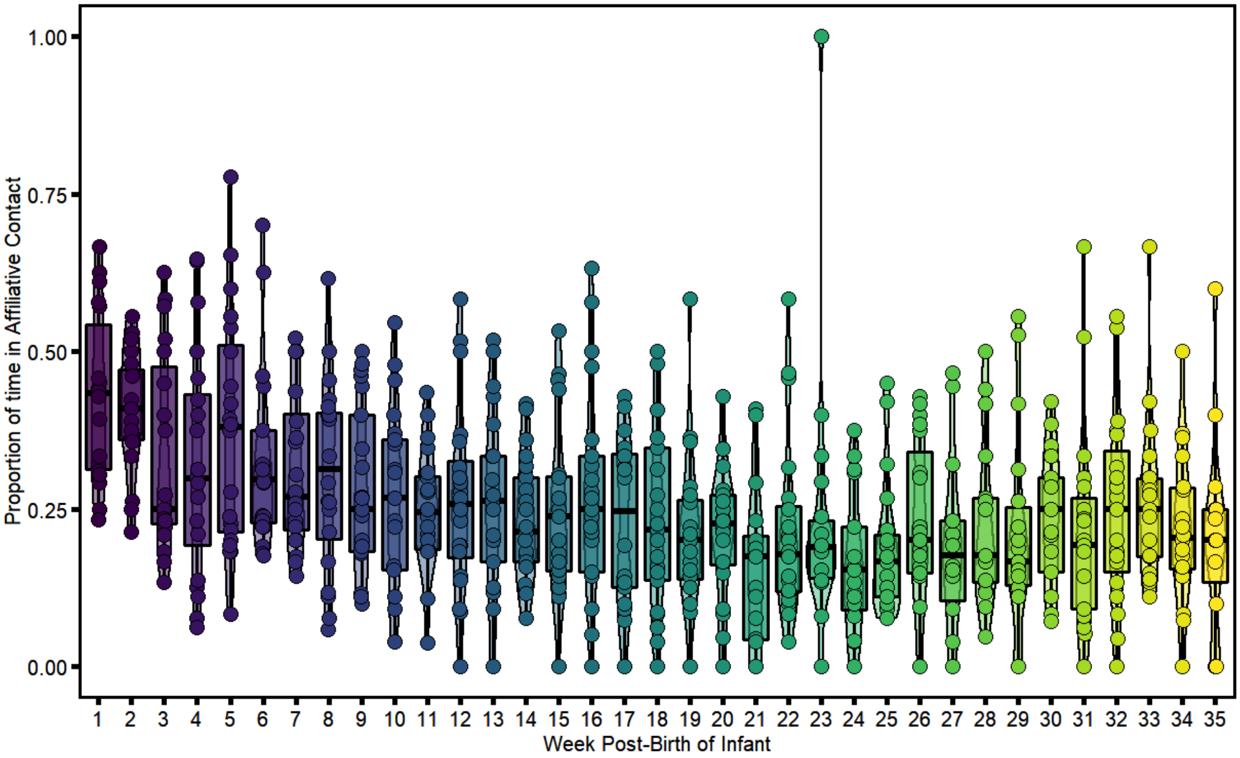

Proportion of time in Affiliative Contact
0.8
0.6
0.4
0.2
8-5 mo pre
4-0 mo pre
0-4 mo post
5-8 mo post
4-Month Periods Pre- and Post-Birth of Infant

Figure 2



Proportion of time in Affiliative Contact
0.5
0.4
0.3
0.2
0.1
8-5 mo pre
4-0 mo pre
0-4 mo post
5-8 mo post
4-Month Periods Pre- and Post-Birth of Infant

Figure 3


Proportion of time in Affiliative Contact
0.00
0.25
0.50
0.75
1.00
1
2
3
4
5
6
7
8
9
10
11
12
13
14
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34
35
Week Post-Birth of Infant

Figure 4

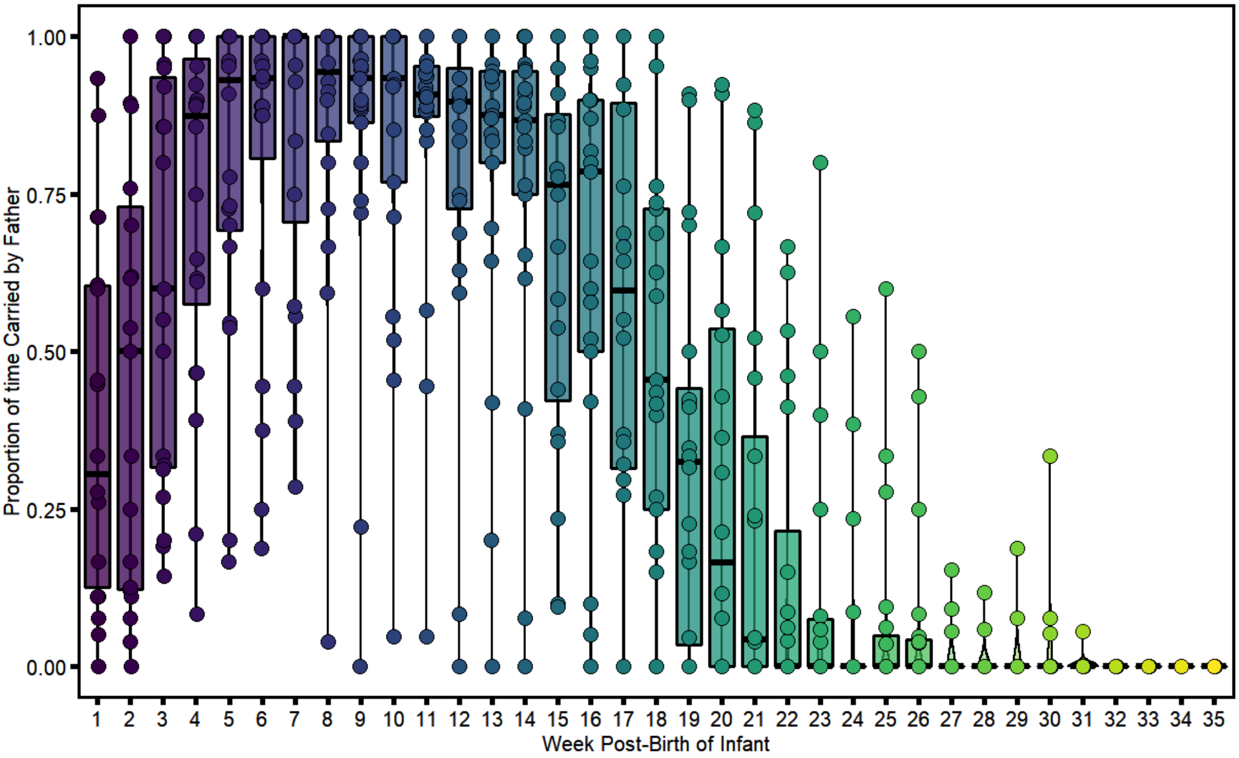
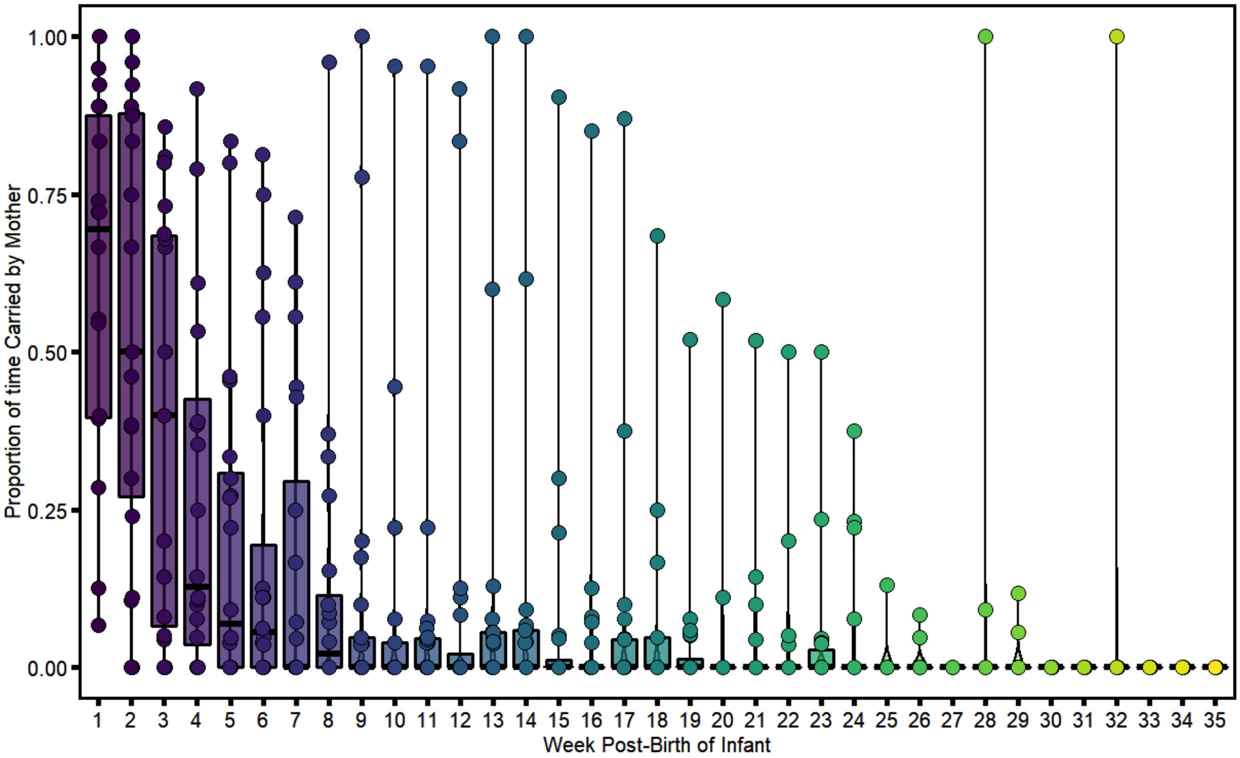

1.00
0.75
0.50
0.25
0.00
Proportion of time Carried by Father
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Week Post-Birth of Infant

Figure 5


Proportion of time Carried by Mother
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
6
7
8
9
10
11
12
13
14
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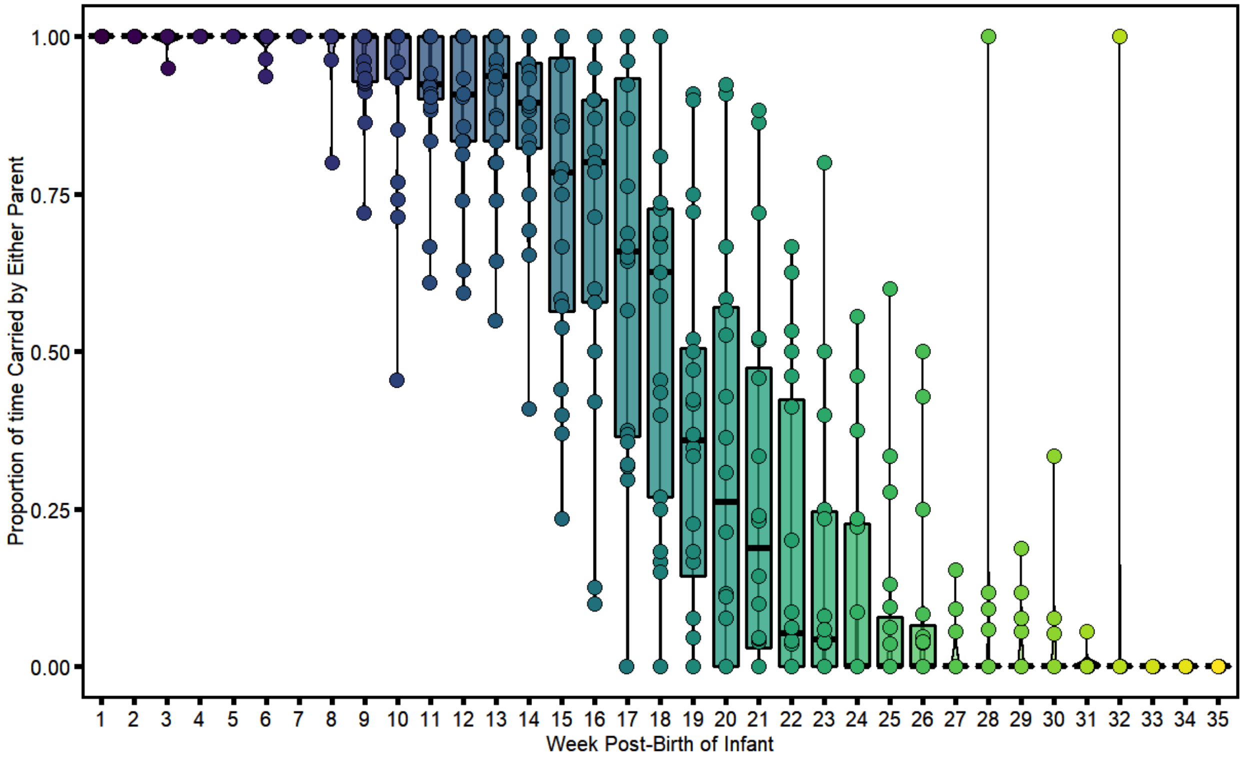
34
35
Week Post-Birth of Infant

Figure 6


Proportion of time Carried by Either Parent
1.00
0.75
0.50
0.25
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
Week Post-Birth of Infant