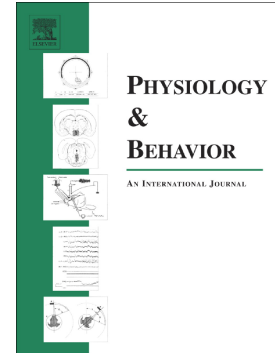


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Long-term-stability of relationship structure in family groups of common marmosets, and its link to proactive prosociality

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**Title:** Long-term-stability of relationship structure in family groups of common marmosets, and its link to proactive prosociality

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## Abstract

Cooperatively breeding, group-living common marmosets show differentiated relationships, where more strongly bonded dyads within a group engage more in affiliative interactions than less strongly bonded ones. Intriguingly, recent results suggest that strong bonds do not only occur between breeding partners but between individuals from any sex or status, and that strong-bond partners exhibit correlated oxytocin fluctuations (dyadic oxytocin synchrony, OTS) over a period of six weeks. To date, it is unclear whether such relationships are stable over time and whether they are also reflected in higher partner-specific proactive prosociality. To assess the long-term stability of the relationship structure of common marmoset family groups, we investigated whether hormonal and behavioral markers of group structure (dyadic OTS, dyadic affiliation, and individual group integration) in common marmoset families remained stable over a period of six months. We collected baseline urinary OT and social behavior of 36 dyads from three family groups in a non-reproductive period (period A), and again six months later, around the birth of new infants (period B). Patterns of dyadic OTS, dyadic affiliation, and individual group integration were consistent between the two study periods. Oxytocin data from a fourth group (10 dyads), collected in two non-reproductive periods separated by a period of more than five years, could replicate this finding. Furthermore, OTS was also correlated with proactive prosociality that was assessed experimentally for 38 dyads during an earlier study. These results suggest that differentiated relationships are stable over time, even between group members other than the breeding pair, and that more strongly bonded partners also show higher levels of proactive prosociality. Future studies are necessary to identify whether these relationships have an adaptive function, perhaps with regard to positive consequences on cooperativeness.

**Key words:** common marmoset, differentiated relationships, dyadic oxytocin synchrony, group stability, proactive prosociality

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## 1. Introduction

Cooperatively breeding callitrichid monkeys like common marmosets live in relatively small and cohesive family groups, in which breeding and non-breeding group members cooperatively care for dependent offspring. This lifestyle and the joint care for infants are associated with high within-group affiliation, and increased social tolerance and prosocial motivation towards other group members [1-3], even though episodes of intense conflict also occur, in particular when competing for breeding positions [4].

Marmoset families are typically composed of parents (breeders), adult offspring, and non-adult offspring [5]. Breeders are socially most central, as they are the most frequent grooming partners [6] and in closer proximity to most other group members, compared to adult offspring who often occupy more peripheral positions [5]. The relationships of breeding pairs are relatively well studied and described as socially monogamous [7, 8]; but see [9, 10], whereas less is known about the relationship structure among other group members in marmoset family groups.

Bonding and positive partner-specific interactions have an adaptive value in many primate species. For instance, reliable bonds among primates have been associated with increased fitness and health (reviewed in [11-13], e.g. in pigtail macaques [14] and baboons [12]), with increased post-conflict reconciliation in pigtail macaques and chimpanzees [15, 16], with higher social rank and the possibility of rank changes due to coalitionary support (reviewed in [17, 18], e.g. in chimpanzees [19, 20] and macaques [21]), and with increased cooperation in chimpanzees, e.g. during hunting [22], food sharing [23], and territory defense [24]. Indirectly this is associated with higher reproductive success, e.g. in female baboons [25-27] and humans [28], and in male macaques [29], chimpanzees [30], and baboons [31].

The strong bonds of callitrichid breeders is consistent with their cooperation in infant care. Furthermore, breeders often groom their offspring more than vice versa, suggesting that

breeders may specifically use grooming as incentive for helpers to stay in the group [32-34]. However, recent evidence also suggests that grooming patterns in marmosets are more dyad-specific and that individuals have preferred grooming partners in their group [35]. Strong bonds thus not only occur in breeding dyads, but also in breeder-helper and helper-helper dyads.

Intriguingly, more affiliative partners in marmoset groups also show synchronized fluctuations of urinary oxytocin (OT) levels over time [35]. These findings and previous results from chimpanzees [36, 37] indicate that OT, a hormone crucially involved in the regulation of mother-infant and sexual bonding, is also important for non-sexual bonding in primates. In chimpanzees, affiliative and prosocial interactions between strong-bond partners elicited higher OT responses in the involved partners than interactions between non-bond partners [36, 37]. Over time, such effects should result in dyadic OT synchrony, since two strong-bond partners will show high OT levels after days when they engaged a lot in affiliative interactions with each other, but lower OT after days when they did less so. Accordingly, in our previous study [35], we found high OT synchrony over time in marmoset dyads with particularly high levels of affiliation but no or a negative correlation of longitudinal OT fluctuations in dyads with lower levels of affiliation. A direct link between absolute individual OT levels and the overall amount of affiliation exchanged was not captured in this dataset, presumably because we investigated average baseline OT levels but not individual OT responses related to specific grooming events.

In group-living marmosets, reliable bonds may be particularly valuable in the context of cooperation during the joint care for infants. Joint care-taking involves complex coordination of infant-transfers and carry-shifts (taking turns during infant-carrying) between care-takers, and it requires increased tolerance among care-takers in the proximity of young infants. If so, the relationship structure of the group, detected during a period without

dependent offspring, should remain stable during reproductive periods, when dependent offspring are present in the group.

Relationships between breeders and helpers may also result in longer periods of acceptance of the helpers in the natal group, or increased allomaternal care by helpers [38, 39]. Strong bonds between same-sex helpers may furthermore provide benefits during dispersal [10, 40]. In all three cases, differentiated relationships as described in Finkenwirth et al. [35], and thus group structure, are predicted to be stable over time rather than a short-lived transient phenomenon.

The aim of this study was to further investigate the nature of dyadic marmoset relationships, with regard to their stability over time, and with regard to whether they are also reflected in experimentally assessed proactive prosociality. We assessed the stability of the relationship structure in common marmoset family groups based on patterns of dyadic OT synchrony (OTS) and affiliative interactions. To do so, we studied three marmoset groups (14 individuals) in a non-reproductive period (period A) and again six months later, around the birth of new infants (period B). In both sampling periods, we measured longitudinal urinary OT levels to assess dyadic OTS, and conducted focal observations of all social behaviors with other group members to assess dyadic affiliation and individual group integration, i.e. the sum of all dyadic affiliation that an individual shared with other group members. Additional dyadic OTS data were available from a fourth group (group Vreni, 10 dyads) that was monitored in a different study context during two non-reproductive periods separated by 5.5 years. Our previous results suggested that higher positive dyadic OTS is associated with stronger bonds in group-living marmosets [35]. To assess the stability of these bonds, we tested the consistency of both dyadic OTS and dyadic affiliation as well as individual group integration over time. Based on the assumption that strong bonds are functional, perhaps because they facilitate cooperation in infant-care, result in longer periods of acceptance of the helpers in the natal group, or may provide benefits during dispersal, we predicted patterns of dyadic OTS, dyadic affiliation, and individual group integration to be correlated between

study periods A and B. Furthermore, we also investigated whether dyads with high OTS not only engaged in higher levels of dyadic affiliation but also showed higher levels of experimentally assessed proactive prosociality, and therefore a stronger disposition to engage in cooperative activities. We did this by a post-hoc analysis of previously collected prosociality data [41] from group four and group five (Kalium) for which prosociality and hormone data were available (total N=38 dyads).

## 2. Methods

### 2.1. Study animals

A total of five marmoset family groups including 28 adult individuals (15 females and 13 males, 46 dyads) were part of this study. For the first three groups (Lancia, Mina and Nina), we collected hormonal and behavioral data first in a non-reproductive period (period A, 6 weeks, data from [35]) and second six months later, in a reproductive period (period B, 6 to 12 weeks, starting one week pre-partum), where dependent offspring were present. Group Lancia was sampled twice in sampling period B, over two consecutive reproductive cycles. For the fourth group (Vreni), hormonal data from two sampling periods were available as well, but no infants were born during the second period. In the fourth group, the gap between sampling periods A (Sept 2007) and B (Feb 2013) was 5.5 years. Individual Vreni died before the second sampling in period B. For the fifth group (Kalium), hormonal data were only available from period A (Sept 2007), collected simultaneously with group Vreni. Furthermore, for both groups four and five, we used experimentally assessed dyadic proactive prosociality data, that were collected approximately ten month before the first OT sampling in 2007 by Burkart et al. [41]. Individual group membership, name, sex, and status of all adult



marmosets that were part of this study, as well as the sampling protocols for behavioral data and proactive prosociality, and the order and date of sampling events in study periods A and B are listed in supplementary tables S1-A and B.

All groups were housed at the Primate Station of the University Zurich in standardized enclosures (depending on group size, one or multiple basic cage units; each measuring 2.4 m height x 1.5 m depth x 0.8 m width), that were connected to spacious outdoor areas and equipped with a sleeping box, a water dispenser, several wooden climbing structures, an infrared lamp and a mulch floor. Indoor enclosures provided natural light and additional artificial light on a 12 h/12 h light–dark cycle and UV light (300W). The animals received a vitamin and calcium-enriched porridge in the morning, fresh fruits and vegetables over midday, as well as gum and mealworms in the afternoon. Water was always available *ad libitum*. Each enclosure was connected to a special cage unit that was only accessible in the morning during urine collection. On urine sampling days, mealworms were fed as reward in the urine cages in the morning instead of afternoon (see Finkenwirth et al. [35] for details).

## **2.2. Behavioral observations**

Behavioral observations of the groups Lancia, Mina and Nina in period A and B (N=36 dyads) were conducted two to three times per week on alternating days, followed by urine sampling in the next morning. We recorded all social interactions, including mutual grooming (picking the fur or skin of a partner with hands or mouth) and huddling (resting in direct body contact). Agonistic behavior was too rare to be included in the analysis. In period A, behaviors were recorded as all occurrences based on continuous observations for 10 minutes per individual (40 to 60 minutes per group, see also [35]), whereas in period B, we scanned all individuals in a group every five minutes over 120 minutes (24 scans per observation day and group). All observed behaviors were corrected for observation time or

the number of scans, respectively. For each dyad, mutual grooming and huddling was combined into one value of dyadic affiliation by summing up the observed frequencies of mutual grooming and huddling. Furthermore, all dyadic affiliation measures were ln-transformed to reach normality and z-transformed prior to analyses in order to eliminate potential variation emanating from the different recording methods. Individual group integration was calculated as the sum of all dyadic affiliation values that an individual shared with other group members. We did not standardize the data for group size, since all groups were very similar in size and we found no influence of group size on dyadic affiliation or individual group integration.

Dyadic proactive prosociality was assessed experimentally for groups four and five (N=38 dyads) by Burkart et al. [41] in summer 2006. This was done using a food provisioning test, in which a donor could choose to pull an empty tray or a tray containing food for a recipient partner [41]. The test consisted of two social test sessions with the partner present and one non-social control session that was conducted between the two test sessions. The three sessions were usually conducted on three subsequent days. Each session included six successive test trials and three motivation trials. The donor's dyadic prosocial tendency was estimated as the percentage of pulling for the recipient in the test sessions minus in the control session. In each dyad, the donor was the older individual, and in the breeder-breeder dyad, the donor was the male breeder.

### **2.3. Urinary oxytocin detection**

We collected baseline morning urine samples for the detection of individual OT levels two to three times per week (alternating groups between two and three sampling days per week) during each sampling period over six consecutive weeks in study period A and over six

to 12 consecutive weeks in period B, starting one week prior to birth. Urine samples were always collected in the morning, following observation days.

OT levels from study periods A and B were measured using the same ELISA technique in the Assay Services Unit of the Wisconsin National Primate Research Center in Madison (WNPRC), Wisconsin, and the endocrinology laboratory of the department of Primatology at the Max Planck Institute of Evolutionary Anthropology (MPI-EVA), Leipzig, Germany, respectively. Mean individual urinary OT levels from both sampling periods are presented in supplementary table S1-A. The urine sampling and OT detection procedures are described in detail in [35, 42]. After collection, urine samples were immediately stored in 1 ml aliquots at  $-20^{\circ}\text{C}$  until measurement. For OT detection, samples were thawed, vortexed, and extracted using solid phase extraction columns (Macherey-Nagel, 55–150 mm, WAT023501). Extracted urine samples were measured in duplicates at a volume of 200  $\mu\text{l}$  according to the directions provided with the assay kit (Assay Designs, Cat no. 901-153). The assay standard curve ranged from 6 to 1000 pg/ml and assay sensitivity was 6 pg/ml. Mean intra-assay coefficients of variation and inter-assay coefficients of variation during the measurements at the WNPRC and the MPI-EVA were 5.85 % and 16.11 %, and 9.67 % and 15.87 %, respectively. OT levels from groups four (Vreni) and five (Kalium) (both part of the dataset from Burkart et al. [41]) were measured in the Institute of Physiological Chemistry of the University Leipzig, Germany, using an in-house ELISA method developed by Einspanier et al. [43]. Mean intra-assay coefficients of variation and inter-assay coefficients of variation of this assay were 11.8 % and 15.2 %. Prior to statistical analysis, all OT concentrations were corrected for creatinine levels to control for variable urine concentration and ln-transformed to reach normal distribution.

Dyadic OTS was calculated as the correlation coefficient of two individuals' longitudinal urinary OT levels [35] for all within-group dyads in each sampling period (total  $N=75$  dyads from groups Lancia, Mina, Nina, Vreni, and Kalium), based on  $N = 8$  to 20 OT

values per dyad. The question whether peripheral OT levels are meaningful estimators of central OT effects related to social behavior and bonding has extensively been discussed in [35, 37, 42, 44]. Using OT data from two different assays is legitimate, since our analyses are only based on relative measures (dyadic OT synchrony) but not on absolute individual OT levels. Hence, potential assay-specific variation in absolute OT values, resulting from different assay binding properties, is not expected to affect correlational values. This issue has also been discussed in detail in Finkenwirth et al. [35].

#### 2.4. Statistics

The aim of the study was to assess the stability of hormonal and behavioral indicators of differentiated relationships in marmoset family groups, including dyadic OT synchrony, dyadic affiliation, and individual group integration (individual sum of total affiliation with other group members), between two sampling periods A and B, and to test the link between dyadic OTS and proactive prosociality. To do so, we first tested the absolute changes in these variables between periods A and B using t-tests for repeated measures.

To assess the stability of dyadic OTS and dyadic affiliation, we used permutation-based linear model analyses (R version 3.0.3: lmerPerm, exact permutation analysis). Both variables were tested independently in separate models. A permutation step was included in the models to control for dependencies due to individuals being sampled repeatedly or as part of multiple dyads in a group, and thus, to control for pseudo-replication across periods and across different reproductive cycles. To do so, dyadic OT synchrony (model 1) and dyadic affiliation (model 2) in period A and B were resampled randomly across groups. Cohen's  $f^2$  was calculated as effect size measure for all permutation linear models.

First, in model 1a, we tested whether dyadic OTS (for 36 dyads from groups Lancia, Mina, and Nina) from period A (OTS-A) was correlated with dyadic OTS from period B

(OTS-B). OTS-B was the dependent variable, and OTS-A, dyad type, and the interaction OTS A\*dyad type were included as fixed factors. To corroborate model 1a, we used dyadic OTS data from the additional group (Vreni) that were collected in a different study context without dependent offspring being present. In model 1b, OTS stability was tested across the combined dataset including all 46 dyads from the four groups.

Second, in model 2, we tested whether dyadic affiliation (for 36 dyads from groups Lancia, Mina, and Nina) in period A (affiliation A) was related to affiliation from period B (affiliation B). Dyadic affiliation B was the dependent variable, and dyadic affiliation A, dyad type, and the interaction dyadic affiliation A\*dyad type were included as fixed factors.

Third, in model 3, we assessed the stability of individual group integration (for 14 individuals from groups Lancia, Mina, and Nina) from period A to period B, using linear mixed-effect model analysis with restricted maximum likelihood estimation (R version 3.1.0, lme package). Individual group integration from part B was the dependent variable, whereas group integration from part A, sex, and status were included as fixed factors, and individual nested in family group was used as random factor. A marginal  $R^2$  was calculated as effect size measure for model 3.

Finally, we analyzed whether OTS was not only related to dyadic affiliation but also to proactive prosociality. To do so, we performed an additional permutation-based linear model to test the link between dyadic OTS (from period A) and proactive prosociality using the data from Burkart et al. [41] for a total of 38 dyads from groups four and five. The model included dyadic proactive prosociality as dependent variable and dyadic OTS and dyad type as fixed factors.

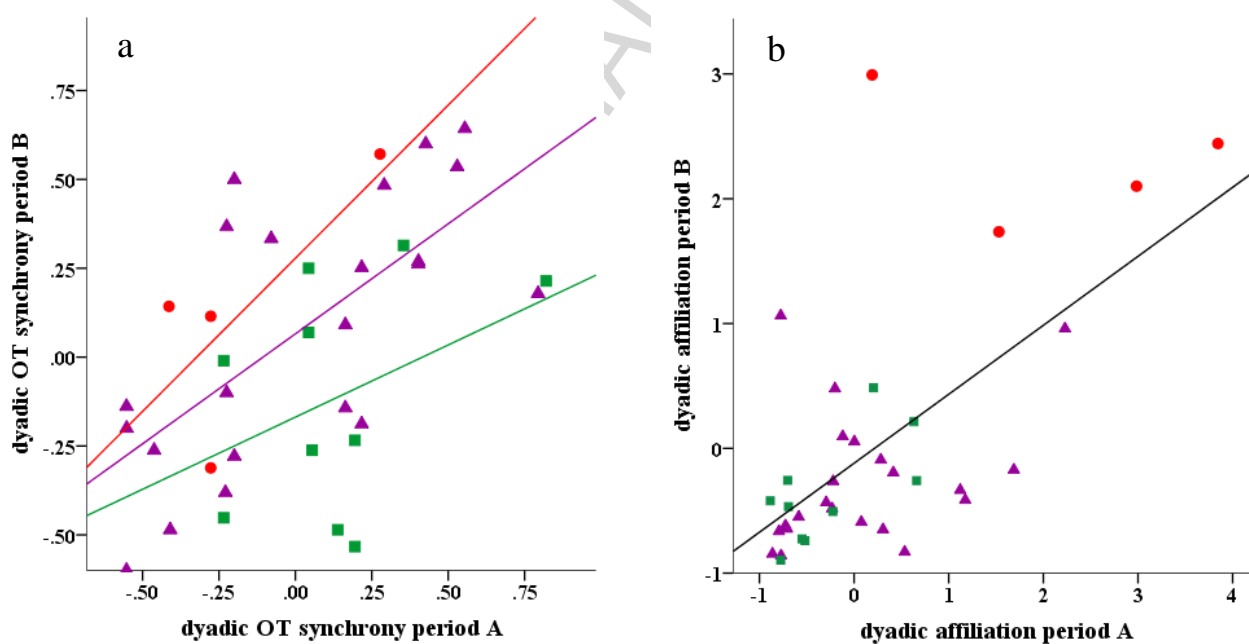
The best-fitting models were selected based on the Akaike information criterion (AIC) estimation for all permutation based linear models and mixed-effect model analyses. Approximate normality and homogeneity of residuals was assessed visually based on residuals plotted against fitted values and qq-plots. All model assumptions were met.

### 3. Results

First, we analyzed whether dyadic OTS patterns remained stable over six months and across reproductive context between sampling periods A and B. Overall, dyadic OTS values did not differ between sampling periods A and B ( $t(35)=-0.67$ ,  $p=0.507$ ,  $d=-0.11$ ). Assessing the stability of OTS values over time revealed a positive link between OTS from periods A and B (Table 1, model 1a, linear model estimate = 0.54,  $p = 0.014$ , Cohen's  $f^2=0.22$ ). This link was strongest and significant in breeder-helper dyads, followed by helper-helper and breeder-breeder dyads (Figure 1a). However, note that the sample sizes (especially for breeder-breeder and helper-helper dyads) are quite small to assess the effects independently. The fact that OTS stability was strongest in breeder-helper dyads is also reflected in a positive influence of dyad type, specifically of breeder-helper dyads, in model 1a. When combining the current dataset with OTS data from group four (Vreni), that was observed in a different study context and in two periods without dependent offspring, we found an even stronger link between dyadic OTS values from sampling periods A and B (Table 1, model 1b, linear model estimate = 0.63,  $p = 0.007$ , Cohen's  $f^2=0.21$ ). This suggests that dyadic OTS patterns are stable over a period of six months (groups one to three) and, potentially, even over a period of more than five years (group four). However, the possibility of long-term stability needs to be treated with caution, since the data also show considerable variation and they are limited to a relatively small sample size.

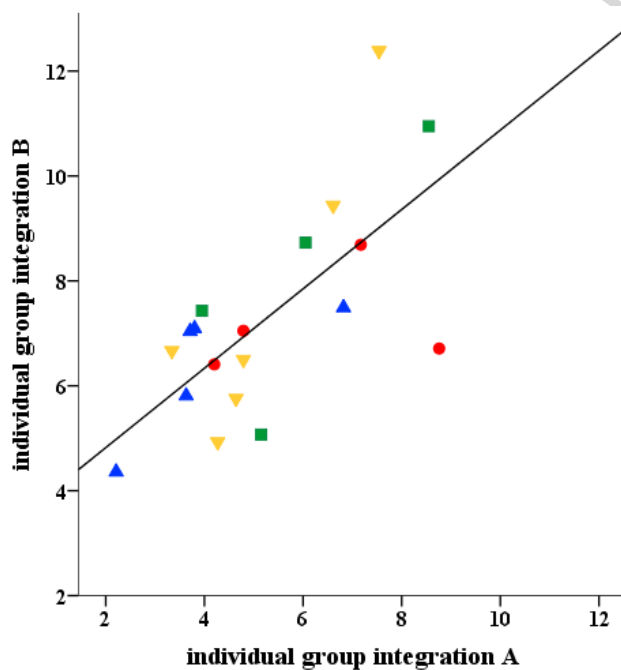
Second, we analyzed whether patterns of dyadic affiliation remained stable over six months and across reproductive context between sampling periods A and B (groups one to three). Overall, dyadic affiliation values did not differ between periods A and B ( $t(35)=-1.40$ ,  $p=0.171$ ,  $d=-0.23$ ). When assessing the stability of dyadic affiliation over time, we found a

significantly positive link between dyadic affiliation values from periods A and B (Table 2, model 2, linear model estimate = 0.50,  $p = 0.016$ , Cohen's  $f^2=0.58$ ). Furthermore, we found an effect of dyad type, reflecting that absolute affiliation levels were lower in breeder-helper and helper-helper dyads compared to breeder-breeder dyads, as illustrated in Figure 1b and described earlier in [35]. Furthermore, we found an interaction effect between dyad type and affiliation from period A. We interpreted this findings in such a way that dyadic affiliation decreased in period B relative to period A in helper-helper dyads as well as some breeder-helper dyads. This may indicate that relatively more affiliation was exchanged in the context of infant-care in part B, especially in those dyads that included the main care-takers – namely breeders and only some male helpers.



**Figure 1: Stability of dyadic OT synchrony and dyadic affiliation over time.** Dyadic OT synchrony (a) (within-dyad correlation of longitudinal OT levels) and dyadic affiliation (b) (z-score of the sum of mutual dyadic grooming and huddling) are stable between study parts A and B across breeder-breeder (red circles), breeder-helper (purple triangles), and helper-helper dyads (green squares). (double column fitting image)

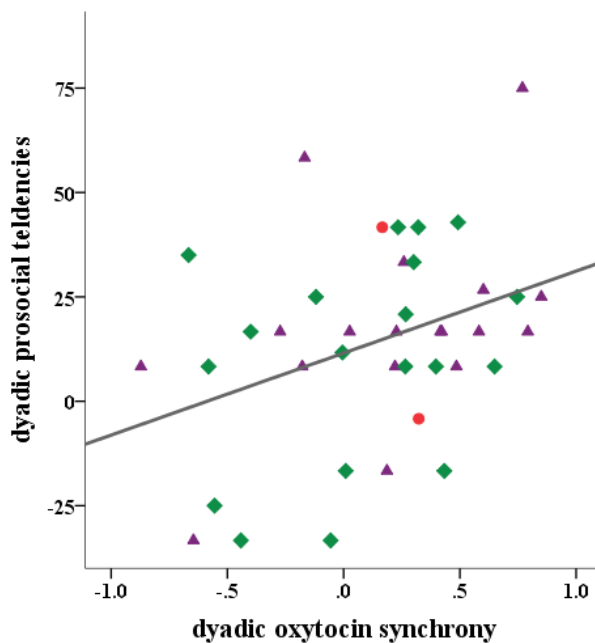
Third, we tested the stability of individual group integration over time. Individual group integration increased for all individuals between sampling periods A and B (Figure 2,  $t(18)=5.76$ ,  $p<0.001$ ,  $d=1.32$ ). This indicates that, overall, more affiliative behavior was exchanged among the members of a group when infants were present. Nevertheless, individual group integration remained stable between study periods A and B, as evident in the positive effect of group integration A on group integration B (Table 3, model 3, linear model estimate = 0.80,  $p = 0.039$ , marginal  $R^2=0.41$ , and Figure 2). Sex or status had no effect in this model. This finding suggests that, despite significant social changes (the birth of new offspring), individuals maintain their relative social integration (characterized by the sum of their total affiliative interactions) in the group.



**Figure 2: Stability of individual group integration over time.** Individual group integration (In sum of total affiliation with other group members) is stable between study periods A and B across breeder females (red circles), breeder males (green squares), helper females (orange triangles), and helper males (blue triangles). (single column fitting image)



Finally, we tested whether dyadic OTS was not only correlated with dyadic affiliation, assessed through behavioral observation (as shown in [35]), but also with dyadic proactive prosociality. We therefore performed a post-hoc analysis on dyadic OTS from period A (collected in summer 2007) and the prosociality dataset in Burkart et al. [41], sampled in summer 2006. We found a significant positive link between dyadic OTS and dyadic prosocial tendencies (Table 4, model 4, linear model estimate = 18.80,  $p = 0.025$ , Cohen's  $f^2=0.23$ , Figure 3). Dyad type had no influence in this model. Although this link is significant, its interpretation requires caution, since the data were not collected prospectively.



**Figure 3. Positive link between dyadic OT synchrony and dyadic prosocial tendencies.**

Dyadic OT synchrony is positively linked to dyadic proactive prosociality (percentage of pulling for the recipient in the test session minus control session) across 38 dyads that were tested in [41] (breeder-breeder (red dots), breeder-helper (purple triangles) and helper-helper dyads (green squares)). (single column fitting image)

#### 4. Discussion

In this study, we investigated whether hormonal and behavioral indicators of differentiated relationships in marmosets are stable over time and context (the birth of infants), and whether these relationships are also reflected in higher levels of experimentally assessed partner-specific proactive prosociality. Such stability is expected if these relationships are indeed adaptive for group-living marmosets.

First, we investigated whether hormonal and behavioral indicators of differentiated dyadic relationships in group-living marmosets remained stable between a non-reproductive and a reproductive sampling period, separated by six months. With regard to hormonal indicators of dyadic relationships, we found that patterns of dyadic oxytocin synchrony (OTS), which are characteristic of highly affiliative dyads [35], were stable over a period of six months, and presumably up to 5.5 years (based on data from one additional group). In the smaller data set, stability of dyadic OTS was only significant in breeder-helper dyads, but the effect was highly significant across all dyad types when an additional group was included and increased the statistical power of the analysis. These results are consistent with the finding that within the same observation period, OTS is linked to dyadic affiliation in all types of dyads [35]. Notably, the two sampling periods of the additional group were separated by 5.5 years, suggesting long-term stability of at least some dyadic relationships. Peripheral OT measurements in the present study were also affected by the birth of infants [42], most likely due to reproduction-related OT functions, especially with regard to parturition and lactation in breeding females [45, 46], and related to infant-directed behaviors during care-taking [47]. After the birth of infants, absolute OT levels increased in all group members, and some variation in OT was directly linked to care-taking activities, in particular infant-licking and proactive food sharing [42]. Nonetheless, our present results show that dyadic OTS patterns are not completely overshadowed by the birth of infants in period B. This indicates that despite the presence of infants and engagement in care-taking, partner-specific OT responses persist and continue to reflect dyadic partner preferences.

With regard to behavioral indicators of dyadic relationships, we found that dyadic affiliation was also stable over six months (not tested for longer periods). This result was strongly driven by breeding pairs who exchange the highest level of affiliation. In breeder-helper and helper-helper dyads, the results also indicate relative changes in dyadic affiliation values between the non-reproductive and the reproductive sampling period. This effect was interpreted as an increase of dyadic affiliation in those dyads that were mainly involved in care-taking and thereby presumably exchanged more affiliation with their current cooperation partners. In contrast, affiliation may decrease in dyads that were less involved in infant-care. More detailed analyses of such potential changes are limited in our dataset due to small sample size, but they should be addressed in future studies. Additionally, to better assess the influence of the presence of young infants on patterns of dyadic affiliation, we investigated the stability of a second relationship measure, i.e. individual group integration (individual sum of total affiliation with other group members). This measure reflects the overall structure of relationships within a group. Individual group integration, and thus overall relationship structure, turned out to be highly stable in all classes of individuals between the two sampling periods, and integration values were generally higher when infants were present. This indicates that overall group cohesion generally increases when infants are present, even though intensified affiliative interactions may not primarily be exchanged between preferred partners, because they may not always be available, but rather in the context of joint infant-care.

Together, these results suggest that hormonal and behavioral indicators of differentiated relationships are indeed stable over at least six months in common marmoset family groups. The pattern is most consistent for breeder dyads, but also detectable in breeder-helper and helper-helper dyads. This is expected since the role of helpers, but not of breeders in a group may eventually change over time [48].

Second, we investigated whether dyadic OTS was not only correlated with spontaneously occurring affiliative behavior, as shown in [35], but also with experimentally assessed proactive prosociality. To do so, we performed a post-hoc analyses of prosociality data, collected in a different context [41], for the dyads from groups four and five, for which hormonal data were also available (collected approximately 10 months after the prosociality tests). We found indeed a positive correlation between OTS and proactive prosociality, albeit not a very strong one. This is not unexpected, since the data were collected with a considerable time lag. Dyad type had no influence in this correlation. The effect may represent a group-specific idiosyncrasy, or an artifact of the fact that behavioral measures of prosocial preferences can only be rough estimates. Prospective studies will be required to further validate this finding.

Taken together, our results arguably suggest that differentiated relationships in marmoset family groups are more than short-lived, transient phenomena, but that they are stable (potentially even over periods of several years) and also positively associated with partner-specific prosociality. The ultimate function of these relationships remains to be established, in particular for non-breeder dyads. One possibility is that strong helper-helper relationships may be beneficial during dispersal or that helpers with strong helper-breeder relationships can stay longer in their natal group. The suggestive link with proactive prosociality makes it also likely that they facilitate cooperative interactions. Future studies need to investigate to what extent strong bonds and group integration are indeed conducive for cooperativeness in marmoset groups, and potentially other cooperatively breeding species. A particularly intriguing question is whether bonding and group integration are simply beneficial because they facilitate coordination and cooperation among dyad partners, or whether they are also associated with higher contributions to cooperative group-level activities, such as infant-care [39].

Finally, we also found that the presence of infants was associated with overall increased affiliation in marmoset family groups. The influence of young infants on group dynamics in callitrichids and other non-human primates has been little studied so far. Our present results show that marmoset groups become more cohesive when infants are born, perhaps due to the necessity to jointly care for the infants. However, in callitrichids, reproductive competition among females may also lead to increased aggression and infanticide, and may thus be the cause of group separations or expulsions of single group members [49, 50]. In other non-human primates, mothers are usually the sole care-takers and may therefore be cautious to shield their babies from attacks or intense contacts with the group. In some species, infant-access may be traded as a commodity for grooming among females [51-54], which may also shape future relationships between the involved individuals, and may put mothers temporarily in more central social positions in their groups. High relationship quality and bond strength among females, in turn, also benefits the infants' survival [25] and determines the time that non-mothers are allowed to handle babies [55]. Nonetheless, an effect of increased cohesion at the group level may only be expected in cooperatively breeding species like marmosets but not in species without allomaternal care. Comparative studies are necessary to test this prediction.

## 5. Conclusion

The results of this study suggest that, in common marmoset family groups, dyad-specific patterns of dyadic OT synchrony (OTS), dyadic affiliation, and individual group integration are stable over a period of at least six months, and likely over five years. The presence of infants did not change the relative dyadic relationship patterns in the group but, overall, groups with infants were more cohesive than without infants. These results suggest that affiliative interactions among marmoset group members are not exchanged randomly, but

that individuals have preferred partners with whom they interact more closely on a regular basis. Over time, this seems to create a stable structure of differentiated relationships in marmoset groups that is also characterized by stable individual social positions and higher levels of experimentally assessed prosociality in stronger bonded partners. Future studies should investigate whether differentiated and stable relationships in marmoset groups also hold an adaptive value, e.g. with regard to positive effects on individual cooperativeness of marmoset care-takers during the joint care for infants.

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## Tables

**Table 1: Stability of dyadic OTS.** Permutation-based linear model results table for the analysis of the link between dyadic OTS from period A and period B (model 1a: groups 1-3 with a delay of 6 months between sampling both periods; model 1b: including group 4 with a delay of 5.5 years). Dyad type with the coding BB (breeder-breeder), BH (breeder-helper), and HH (helper-helper) was included as fixed factor. Parameter estimates: factor levels with bars are compared with remaining factor levels. Bold:  $p < 0.05$ .

Model	Dependent variable	Model factor	F	df	p-Value	Factor level	Estimate	p	
1a	OTS B	OTS A	<b>6.46</b>	<b>1</b>	<b>0.015</b>		<b>0.54</b>	<b>0.014</b>	
		dyad type		2.15	2	0.129	BH	<b>0.22</b>	<b>0.041</b>
							HH	-0.04	0.823
							BB	-	-
		OTS A*dyad type		1.21	2	0.308	OTS A*BH	0.58	0.115
							OTS A*HH	-0.24	0.145
					OTS A*BB	-	-		
1b	OTS B	OTS A	<b>9.21</b>	<b>1</b>	<b>0.004</b>		<b>0.63</b>	<b>0.007</b>	
		dyad type		1.61	2	0.210	BH	0.19	0.435
							HH	-0.05	0.534
							BB	-	-

**Table 2: Stability of dyadic affiliation.** Permutation-based linear model results table of the analysis of the link between dyadic affiliation values (model 2) from part A and part B. Dyad type with the coding BB (breeder-breeder), BH (breeder-helper), and HH (helper-helper) was included as fixed factor. Parameter estimates: factor levels with bars are compared with remaining factor levels. Bold:  $p < 0.05$ .

Model	Dependent variable	Model factor	F	df	p-Value	Factor level	Estimate	p
2	affiliation B	affiliation A	<b>5.87</b>	<b>1</b>	<b>0.022</b>		<b>0.50</b>	<b>0.016</b>
		dyad	<b>3.48</b>	<b>2</b>	<b>0.044</b>	BH	0.77	0.270

	type				HH	-0.77	0.019
					BB	-	-
	affiliation A*dyad type	3.32	2	0.049	affiliation A*BH	-0.47	0.047
					affiliation A*HH	-0.43	0.098
					affiliation A*BB	-	-

**Table 3: Stability of individual group integration.** Linear mixed-model results table of the analysis of the link between individual group integration from periods A (integration A) and B (integration B) (model 3). Status and sex were included as fixed factors, and individual nested in family as random factor. Parameter estimates: factor levels with bars are compared with remaining factor levels. Bold:  $p < 0.05$ .

Model	Dependent variable	Model factor	F	df	p-Value	Factor level	Estimate	t-value	p
		integration A	9.35	1	0.038		0.80	3.04	0.039
3	integration B	status	0.54	1	0.482	helper	0.59	0.73	0.482
						breeder	-	-	-
		sex	0.14	1	0.719	male	0.34	0.47	0.648
						female	-	-	-

**Table 4: Dyadic OT synchrony and prosocial tendencies.** Permutation-based linear model analysis of the link between dyadic OTS (sampling period A) and dyadic prosocial tendencies of 38 dyads from Burkart et al. [41]. Dyad type with the coding BB (breeder-breeder), BH (breeder-helper), and HH (helper-helper) was included as fixed factor. Parameter estimates: factor levels with bars are compared with remaining factor levels. Bold:  $p < 0.05$ .

Model	Dependent variable	Model factor	F	df	p-Value	Factor level	Estimate	p
4	dyadic OTS	prosocial tendencies	4.73	1	0.037		18.80	0.025
		dyad type	0.18	2	0.840	BH	1.71	0.495
						HH	1.38	0.863
						BB	-	-

**Highlights**

- Patterns of dyadic oxytocin synchrony and affiliation indicate differentiated relationships in marmosets
- Dyad-specific patterns of dyadic oxytocin synchrony and affiliation are stable over 6 months
- Individual group integration (affiliativeness with other group members) is stable over 6 months
- The stability of these dyadic and individual variables is undisturbed by the presence of infants
- The results indicate long-term stability of differentiated relationships in marmoset groups

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