# FACTORS AFFECTING THE SUCCESSFUL PAIRING OF UNFAMILIAR COMMON MARMOSET (CALLITHRIX JACCHUS) FEMALES: PRELIMINARY RESULTS

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#### **Abstract**

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Laboratory primates are often housed in same-sex pairs to avoid single-housing and when breeding is to be prevented. However, pair formation is not without risks, as fights and injuries may occur. No data are available on pair formation in female common marmosets (Callithrix jacchus), a species used extensively in laboratories. Therefore, this study focuses on the pairing of unfamiliar common marmoset females, aiming to assess its success rate and whether age can predict the result. Data on the study animals and success of the pairings were extracted from laboratory back-records: a total of 28 pairings was obtained. In addition, behavioural data were collected on six of the 28 pairs. Almost 80% of pairs were compatible beyond one week, and most of the fights occurred well within the first week after pair formation. Pairs in which one of the females was sexually immature (ie < 15 months) were significantly more compatible than pairs in which both females were post-pubertal. First encounters were characterised by sniffing of the unfamiliar monkey. Aggressive behaviours occurred frequently following pair formation but they were unidirectional, and in only two pairs was veterinary treatment required. This study shows that pairing of unfamiliar common marmoset females is a safe practice if one monkey is sexually immature, a result that supports observations of both group and pair formation in other primate species. However, given the potentially detrimental effects of removing young females from their natal groups, we argue that it is preferable to remove two sisters from their natal group when female pairs are required. However, when a single sexually mature female requires a pair mate so as to avoid single-housing and no mature sibling is available, an older, but still sexually immature, unfamiliar female that has had a normal development within the family should be considered as a pair mate.

**Keywords**: animal welfare, colony management, common marmoset, husbandry, pair formation

# Introduction

Group housing is usually the most appropriate housing condition for social primates (Bernstein 1989). Although the procedures for creating new groups are well documented (especially for *Macaca* spp; see eg Bernstein 1989; Hartner *et al* 2001; Westergaard *et al* 

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1999), the artificial formation of groups using adult individuals is not without risks. The animals may be incompatible, and stress, injuries and even deaths may occur (Clarke & Blanchard 1994; Schapiro *et al* 1994). Similar results have been obtained even when monkeys have had a pre-familiarisation period before group formation (Vermeer 1997). However, group formation (of both same-sex and male-female groups) seems to be a safe practise when monkeys are socially experienced juveniles or sub-adults (Wolff & Ruppert 1991).

In captivity it is not always possible to house social species of primates in groups the size of their wild counterparts because of space limitations and/or research procedures (Line et al 1991; Watson 2002). Two widely used housing conditions are single- and pair-housing. Historically, single-housing was considered advantageous in laboratories because it reduced the frequency of trauma and disease transmission, prevented fights, and allowed easy access to animals (Coe 1991). However, a number of studies have shown that singly caged primates show signs of distress and of poor welfare which can even lead to diseases such as psychogenic polydipsia (Stamp Dawkins 1998; Gwinn 1996). Pair-housing represents a good alternative to single-housing. However, it is usually an experimental requirement that breeding is prevented, so if opposite-sex pairs are created, the male is vasectomised. This surgical procedure is invasive and is required only for the husbandry of the monkeys. For this reason, same-sex pairs may be a better alternative to improve the social behaviour of monkeys (Lynch 1998; Schapiro et al 1996). Although some individuals may be injured and aggressively excluded from food in newly formed opposite-sex pairs (common marmoset, Callithrix jacchus: Evans 1983), pairing of unfamiliar monkeys is considered "an effective means of environmental enrichment" (Reinhardt 1997, p 3). Indeed, a growing body of data has pointed out that this is a safe method for macaques, as injuries and signs of distress occur at only a very low rate (Kurth & Bryant 1998; Byrum & Claire 1998; Reinhardt 1998). Both opposite-sex and same-sex macaque pairs have been studied, obtaining a high percentage of success and a low rate of aggression within pairs when certain criteria are followed (Crockett et al 1994; Lynch 1998). Indeed, studies on group and pair formation in macaques (Reinhardt et al 1995; Watson 2002; Wolff & Ruppert 1991) suggest that younger, sexually immature monkeys are subjected to lower rates of aggression than older monkeys, and that a prefamiliarisation period also reduces aggression.

Unfortunately, most of the studies have focused on a restricted number of species, especially of the genus Macaca (see for example Reinhardt 1998), although there are practical implications for the management of other laboratory monkeys. The Medical Research Council (MRC) Unit frequently pairs up common marmoset females as a means of avoiding single-housing and preventing breeding during experimental procedures. Moreover, this practice allows better utilisation of the cage space than does single-housing. At present, no data are available on factors affecting the success rate for pair formation in unfamiliar female common marmosets. In the wild, common marmosets live in family units comprising a breeding pair plus one or more sets of offspring. The younger individuals help the breeding pair to rear the infants (Koenig 1995) and, by doing this, they gain the rearing experience necessary for their own successful breeding in the future (Snowdon & Savage 1989). The dominant/breeding female releases pheromones by means of scent-marking, which suppresses ovulation in female subordinates (Abbott 1987). Therefore, although pairing of females may appear to be a good alternative to single-housing, it forms an unnatural grouping which may affect the psychological well-being of the monkeys. This is particularly true if one considers that common marmoset females are very aggressive towards conspecifics of their own sex (Epple 1970; Rothe & Darms 1993). The hormonal state of

common marmoset females plays an important role in the quality of their first encounters (Epple *et al* 1993). In particular, the rate and direction of aggression between unfamiliar females depend more on the physiological status of the monkeys than on their size (Saltzman *et al* 1996). Given the widespread use of pair-housing of marmosets in laboratories, understanding the factors affecting the compatibility of pair mates is critical in the choice of potential pair mates. Furthermore, correctly interpreting their initial behaviour may prevent harmful and stressful situations for the monkeys.

With this background, the aims of the present study were, first, to assess the success rate of pair formation in female common marmosets; second, to analyse whether age of the marmosets is a factor that affects the success of pairing; and third, to determine how unfamiliar female marmosets interact with each other during the initial stages of pair formation.

#### Methods

# General procedure for pairing unfamiliar marmosets

The research was conducted at the MRC Human Reproductive Science Unit, Edinburgh. All of the monkeys that were paired came from their family groups (group size ranging from six to 12 individuals). The staff paired up an older monkey with a younger one, although the age of the monkeys varied widely depending on the practical needs and research protocols of the laboratory. The staff did not follow the same procedure and criteria for every pairing, and the exact procedure was not noted in the back-records. One or both individuals were transported in a nest box and released, either in an unfamiliar cage, or in a cage previously inhabited by one of them or by both of them (ie the two cages were joined together). There was no prefamiliarisation period. Monkeys were usually paired up between 1000h and 1300h (ie after cleaning and before feeding time), and newly formed pairs were checked daily by the staff, to assess if monkeys were injured and had access to the food dish. Several common marmoset males (living in same-sex or in male–female pairs) were housed in the same rooms where the pairings took place. In this paper, pairings are defined as successful if no bi-directional aggression and/or if no injuries were observed by the end of the study (ie the monkeys had been living together for 13.82 ± 6.78 consecutive months).

### Study animals and housing conditions

Using the MRC Unit back-records, data were compiled on 56 monkeys (without previous pairing experiences) that were paired in the period December 1997 to April 2000. A total of 28 pairings was obtained. Behavioural data were collected on six of these pairs (see below). All of the newly formed pairs were housed in cages of the same size (approximately 55 cm wide × 95 cm high × 110 cm deep) and furnishing (one nest box, two natural branches in different orientations and a water bottle). Each cage had an open tray floor covered with wood shavings. Temperature was maintained at 22–23°C and there was a 12:12 h light:dark cycle. Monkeys were fed once per day (at approximately 1300h) on a mixture of standard primate pellets, together with fresh fruit (eg grapes, apples, oranges, pears and tomatoes) and a special porridge containing proteins, yoghurt, vitamins and minerals. Water was available *ad libitum*.

#### Behavioural data collection

Behavioural data were collected on six pairs. Data were collected for both females simultaneously (focal pair sampling: Huxley 1968 in Lehner 1996) using instantaneous, all

occurrence, and one-zero sampling methods (Altmann 1974; see Table 1 for definitions of the behavioural categories recorded). All of the data were collected on checksheets from a hide with a one-way mirror with which the monkeys were familiar. Data collection started within the first minute after pair formation and the monkeys were observed for the following 30 min. One of the observed pairs showed particularly high levels of agonistic behaviours so that the monkeys had to be separated after 5 min of observation. In order to avoid any effects on the rates of observed behaviours resulting from different time windows, only the first 5 min during pair formation are presented. Data on aggressive behaviour and allo-grooming were also collected from 20 stable female pairs during a concurrent study undertaken at the MRC Unit (Majolo 2001). These female pairs had been paired using the same procedure and had been living together for a considerable period of time, ranging from 61 to 1121 days. The mean age of older monkeys living in stable pairs was  $938.5 \pm 66.4$  days (ie 31.3 months) while that of younger monkeys was 527.7 ± 29.6 days (ie 17.6 months). Data were collected with the same sampling methods and the same procedure as for the newly formed pairs. Data are shown as rate per individual per hour of observation in Table 2; otherwise they are shown as mean scores per pair per hour of observation.

Table 1 Definitions of the behavioural categories recorded (see Stevenson & Poole 1976 for a full description of behaviours).

Behavioural category	Definition
Proximity <sup>a</sup>	The monkey is less than 10 cm from another monkey
Physical contact <sup>a</sup>	Stationary, side to side, body contact with another monkey
Aggressive behaviours <sup>b</sup>	Includes frown, tufts/ears flick or forward, arch bristle locomotion, genital present with tail raised, cackle, cuff, and bite
Submissive behaviours <sup>b</sup>	Includes open mouth, bared teeth, withdrawal gesture
Fear–Alarm <sup>b</sup>	Pilo-tail, sway, tail snake, and see, seep and tsak vocalisations
Scent-marking <sup>b</sup>	The monkey rubs ano-genital area or chest on substrate
$Sniffing^b$	The monkey sniffs the unfamiliar monkey
Allo-grooming <sup>c</sup>	The monkey grooms or is groomed by her social companion using her fingers or mouth

<sup>&</sup>lt;sup>a</sup>Behaviours recorded with instantaneous sampling, 20 s intervals

#### Data analysis

In order to test whether the age of the monkeys was a good predictor of successful pairings, Mann-Whitney U tests were run using alternately the age of older and younger monkeys of each pair, and the difference of age between the two monkeys as the dependent variable, and the results of pairing as the grouping variable (Siegel & Castellan 1988). Two Mann-Whitney U tests were also run to compare rates of aggressive behaviour and allo-grooming between newly formed pairs and stable female pairs. All of the tests were two-tailed and the significance level was fixed at P < 0.05. Missing values were not considered in the data analysis. Means and standard errors are presented in the text. Behavioural data on pair formation were not subjected to statistical analyses because of the small sample size and the presence of many zero scores.

<sup>&</sup>lt;sup>b</sup>Behaviours recorded with all occurrence sampling

<sup>&</sup>lt;sup>c</sup>Behaviour recorded with one–zero sampling, 20 s time intervals

Table 2 Scores per individual and per pair (rate per hour) of newly formed pairs (\*unsuccessful pairing; O, older monkeys; Y, younger monkeys).

Pairs	Cage of pairing originally inhabited by:	Age (days)		Aggressive behaviours		Submissive behaviours		Fear–Alarm		Scent- marking		Sniffing	
		O	Y	0	Y	0	Y	O	Y	o	Y	O	Y
R871–936	0	731	456	9	0	0	15	0	24	12	0	9	15
R927–938	Y	464	406	12	0	0	0	0	0	2	0	10	4
R773–906	O	1325	583	30	0	0	40	0	72	12	3	0	24
R891–960	Both (two cages were joined together)	551	258	20	0	0	30	0	58	6	0	6	5
R905–919	Neither (unfamiliar cage)	562	525	0	103	111	0	114	0	0	39	6	6
Mean per individual	_	726.6	445.6	14.2	20.52	22.2	16.9	22.8	30.8	6.4	8.4	6.2	10.8
Mean per pair	_	586.5		17.4		19.5		26.8		7.4		8.5	
R863–905*	Both (two cages were joined together)	763	559	0	336	228	0	42	0	0	0	12	54
Mean per pair	_	661		167.9		114		21		0		33	

#### Results

#### Success rate

Overall, nine pairs out of 28 were split up because one of the monkeys was subject to intense aggression and/or was injured as a consequence of fighting. When the time between pair formation and separation of the monkeys as a consequence of fights was taken into account, it was evident that a temporal gap existed in the distribution of fights. Sixty-six per cent of the unsuccessful pairs (6/9) were split up within the first week after pair formation, whereas the remaining ones (33.4%) were split up after at least nine months. It is likely that only fights occurring within a short period (ie one week) of pair formation were the direct result of this pairing. Therefore, the initial pairing success rate was 78.6% (22/28) considering only fights occurring within the first week. However, the percentage of successful pairings (67.9%, 19/28) remained at a similar level to the initial pairing success rate even when one considers the whole study period and not just the first week after pair formation.

# The effect of age

The age of older females differed significantly between successful and unsuccessful pairings  $(U=39, n_1=19, n_2=9; P<0.05)$ . The mean age of older females that were successfully paired was  $592.9 \pm 39.4$  days (ie 19.8 months) whereas that of older females that were unsuccessfully paired was  $794.1 \pm 70.6$  days (ie 26.5 months). No significant difference was found for younger monkeys between successful and unsuccessful pairs (successful pairs  $304.1 \pm 27.3$  days [ie 10 months], unsuccessful pairs  $427.1 \pm 85.9$  days [ie 14.2 months];  $U=54.5, n_1=19, n_2=9$ ; not significant).

As stated above, it is likely that only fights occurring within a short period (ie one week) were the direct result of pair formation. Therefore, the same tests were conducted, this time considering as unsuccessful only those pairs that had been split up in the first week after pair formation. The age of the older monkeys was not significantly different between successful and unsuccessful pairings (successful pairs  $636.4 \pm 42.5$  days [ie 21.2 months], unsuccessful

pairs  $735 \pm 92.4$  days [ie 24.5 months]; U = 48,  $n_1 = 22$ ,  $n_2 = 6$ ; not significant). In contrast, the age of the younger monkeys was significantly different between successful and unsuccessful pairings: the younger females were younger in successful (297.5  $\pm$  23.9 days [ie 9.9 months]) than in unsuccessful pairs (512.8  $\pm$  115.2 days [ie 17.1 months]; U = 21,  $n_1 = 22$ ,  $n_2 = 6$ ; P < 0.05). In the three pairs that were separated several months after pair formation, the age of the younger females when fights occurred was 544 days (ie 18.1 months), 590 days (ie 19.6 months), and 540 days (ie 18 months), respectively. Finally, the difference in age between older and younger monkeys was compared in successful and unsuccessful pairings but the result did not reach statistical significance (successful pairs  $288.7 \pm 49.2$  days; unsuccessful pairs  $367.0 \pm 94.7$  days; U = 76,  $n_1 = 19$ ,  $n_2 = 9$ ; not significant). This was also the case even when only the first week after pair formation was analysed (successful pairs  $338.9 \pm 51.8$  days; unsuccessful pairs  $222.2 \pm 83.3$  days; U = 43,  $u_1 = 22$ ,  $u_2 = 6$ ; not significant).

#### Behavioural data

The monkeys on which behavioural observations were carried out were paired successfully in 5/6 cases (83%; ie these monkeys were not separated as a result of aggression and they lived together for a period ranging from 56 to 135 days). Sniffing was the first behaviour displayed by 83.3% (10/12) of the monkeys. In one successful pair, aggressive behaviours and signs of fear or alarm (8.3% each) were the first behaviours displayed. Overall, the percentage of time that the monkeys spent in proximity was very low (ie  $16 \pm 5\%$  of point samples) and physical contact was observed in only one (successful) pair. Social interactions were characterised by sniffing and aggressive/submissive behaviours, whereas allo-grooming was never observed. All of the behaviours recorded (excluding sniffing) showed a clear unidirectional pattern within each pair (ie they were displayed by only one member of the pair; see Table 2). From an animal welfare point of view it is important to assess whether the occurrence of agonistic behaviours and the lack of any amicable behaviour (ie allo-grooming) were temporary effects resulting from the presence of an unfamiliar monkey, or whether they were typical of common marmoset female pairs. Therefore, scores of aggressive behaviour and allogrooming in successful newly formed pairs were compared with scores collected on stable female pairs. Rates of aggressive behaviours were significantly lower in stable female pairs than in successful newly formed pairs  $(3.1 \pm 2.1 \text{ and } 17.4 \pm 9.8 \text{ events per hour, respectively;})$ U = 4,  $n_1 = 20$ ,  $n_2 = 5$ ; P < 0.01) whereas no significant difference was found for allogrooming  $(0.01 \pm 0.00\%)$  and  $0.00 \pm 0.00\%$  of 20 s intervals, respectively; U = 37.5,  $n_1 = 20$ ,  $n_2 = 5$ ; not significant).

Although there are only few data (six pairs), it appears from our results that the location of pair formation had no consistent effect on the success of pairing. Of the five successful pairs for which data are available, three pairs were formed in the cage of one pair member, one pair was formed in a cage unfamiliar to both pair members, and one pair was formed in the cages of both pair members (ie the cages were joined together). The unsuccessful pair was also formed in the cages of both pair members (Table 2).

#### **Discussion**

# Success rate and effect of age

The main conclusion of this study is that pairing unfamiliar common marmoset females is a safe method of pairing and housing. In this study, the monkeys were not familiarised before being paired so that they did not have the opportunity to establish a clear

dominance–subordination relationship before pair formation (Reinhardt 1992). It is well known that common marmoset females are very aggressive towards same-sex conspecifics (Epple 1970). Moreover, same-sex aggression increases if females are in visual contact with common marmoset males (French & Inglett 1989). Therefore, the success rate in this study is high despite the lack of a pre-familiarisation period. Additionally, males were present in the same rooms as the newly formed female pairs (see Methods section), which may also have negatively affected pairing success.

The age of the older monkeys affects the success of pairing in the long term, while that of the younger monkeys affects it in the short term. Overall, these results indicate that younger monkeys are more tolerant towards same-sex conspecifics and/or elicit lower rates of aggression. In particular, pairings in which one of the females is sexually immature are most likely to be successful (sexual maturity is reached at approximately 15 months of age; Stevenson & Rylands 1988; Yamamoto 1993). This suggests that the hormonal state of the females is the main factor affecting the quality of social interactions in female-female encounters, as pointed out in previous studies (eg French & Inglett 1989; Rothe & Darms 1993). Choosing younger monkeys also decreases the chances of aggressive behaviour occurring in both group and pair formation in other species (Hartner et al 2001; Reinhardt et al 1995; Wolff & Ruppert 1991). The occurrence of fights and thus the rate of success may also depend on the procedure used for pairing. It may be that fights occur at a higher rate when pair formation takes place in the cage of one of the monkeys (ie the cage owner may consider the other monkey as an intruder) than when it takes place in a cage unfamiliar to either of the monkeys (ie in a more 'neutral' environment). Unfortunately, cage location of pair formation was not recorded on the back-records, so the relationship between success rate and location could be assessed for only a small sample size. The few data available suggested that the neutral environment was not critical, as three out of five successful pairings were in the cage of one of the pair members.

The age of the younger females in the three pairs that were separated several months after pair formation suggests that the monkeys began to fight when the younger one reached sexual maturity. This may be linked to the presence of common marmoset males in the same rooms; that is, female pairs began to fight once they were both sexually mature and they could smell the presence of conspecific males. Unfortunately, it is not possible to determine whether this factor affected the occurrence of fights among female pairs, for males were present in all of the rooms where female pairs were housed (and therefore it was not an experimental confound). It may be better practice to keep same-sex pairs in same-sex rooms.

The greater success of pairing younger females must be weighed against the welfare implications of removing immature females from their natal groups. Early removal may have many consequences on the behaviour of the females (Tardif *et al* 1984) and the practical and ethical implications have to be considered carefully. Snowdon and Savage (1989) in their review on the welfare of the Callitrichids stated that marmosets and tamarins should be housed with their family for at least two sets of infants in order to gain infant care experience and thus the complete behavioural repertoire of the species. Therefore, removing monkeys from their natal group before ten months of age means that they will have had experience with only one set of infants. None of the paired females was destined for breeding, and hence the importance of learning infant care is less critical in this instance. Nevertheless, a normal development within the family group is important to ensure reliable and valid experimental results. Hence, to avoid single-housing, it is recommended that older, but still sexually immature, unfamiliar females that have had a normal development within the family, should be selected as pair mates.

#### Behavioural data

In each of the six pairs that were the subject of behavioural observation, agonistic behaviours were unidirectional. The preponderance of unidirectional aggression/submission suggests that common marmosets are able to assess their own hierarchical status and that of their social companion immediately on the first encounter. This is probably due to the importance of the hormonal state of monkeys on their hierarchical status (Epple et al 1993). In fact, sniffing was almost always the first behaviour displayed in every newly formed pair. However, other factors (such as size; Saltzman et al 1996) may affect the establishment of hierarchical status. Unfortunately, weights were not available for females so the importance of this factor could not be assessed. As stated by Reinhardt and Reinhardt (2000 p 7), "the compatibility of pair-housed primates is founded on clear-cut dominance-subordination relationships" and thus, from an animal welfare point of view, aggressive displays have to be considered as part of pair formation, even in successful pairings, if they do not degenerate into behaviours that are dangerous for the monkeys (eg physical assaults). In only two of the unsuccessful pairs (using data from the back-records) did fights result in injuries that required veterinary treatment; in the remaining unsuccessful pairs both monkeys displayed aggressive behaviours so they had to be separated.

Kummer (1995) showed that in baboons and in other cercopithecine species, a fixed sequence of behaviours is exchanged between two unfamiliar monkeys when they first meet. Agonistic behaviours come first, and the establishment of social relationships, by means of affiliative behaviours (ie allo-grooming), follows. In this study, common marmosets seemed to follow a fixed pattern of behaviours: sniffing was displayed first, possibly to assess the physiological status of the unfamiliar monkey, then agonistic behaviours were exchanged but no affiliative behaviour was observed, excluding one case of physical contact. This result might be attributable to methodological flaws (eg observation sessions were too short and affiliative behaviours occurred later on the day of pair formation), but it is more likely that affiliative behaviours are exchanged rarely among common marmoset females (Majolo 2001). Our data on stable female pairs suggest that the two monkeys seem to tolerate or ignore their social companion rather than physically interacting. However, no data were collected on singly housed marmosets so it is not possible to examine the welfare implications of single- versus pair-housing. It is likely that in social species such as marmosets, singly housed animals suffer as a consequence of the lack of social interactions, such as huddling in the nest box. Furthermore, it should be noted that olfactory communication is critical in marmosets, and the welfare implications of this aspect of marmoset behaviour are difficult to determine.

#### Animal welfare implications

Many laboratories pair-house same-sex common marmosets for experimental purposes as an alternative to single-housing. This is an unnatural social grouping for this species, and pair formation of unfamiliar unrelated individuals is not without risk. However, despite the findings from this study that there are few observable affiliative interactions between female pair-mates, pair-housing is likely to be a preferable alternative to single-housing in a social species such as this.

This study has shown that age is a critical factor in the successful pairing of female marmosets; the pairs are more stable if one female is sexually immature. However, given that a normal social development is important in generating valid and reliable experimental results, one must be cautious about removing sexually immature individuals from their natal

groups. If there are practical reasons for housing female pairs, then removing mature (> 15 months) same-sex offspring as pairs is a preferred solution to pairing unfamiliar individuals. This recommendation is easy to implement, as common marmosets breed frequently, producing twins or triplets twice a year as a norm in laboratories. However, when a single sexually mature female requires a pair mate and no mature sibling is available, an older, but still sexually immature, unfamiliar female that has had a normal development within the family should be considered as a pair mate so as to avoid single-housing.

A larger data set is required to determine the importance of factors such as cage of pairing, weight of the monkeys and male presence on the result of pairing females. Moreover, to assess the welfare and the level of stress associated with pair formation and housing in various groupings, comparative behavioural and physiological data must be collected on sibling pairs, newly formed pairs and stable female pairs as well as on singly housed monkeys.

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