

## RESEARCH ARTICLE

Laboratory Simulations of Mate-Guarding as a Component of the Pair-Bond in Male Titi Monkeys, *Callicebus cupreus*

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Mate-guarding and territorial aggression (both intra- and inter-sexual) are behavioral components of social monogamy seen in male coppery titi monkeys (*Callicebus cupreus*) both in the field and in the laboratory. Methodology for studying these behaviors in captivity facilitates the translation of questions between field and laboratory. In this study, we tested whether exposure to a mirror would stimulate mate-guarding behavior in male titi monkeys, and whether this exposure was accompanied by hormonal changes. Eight males were exposed to a mirror condition (treatment) or the back of the mirror (control) for five sessions, and behavioral responses were filmed. Blood samples were taken to measure levels of cortisol, oxytocin, and vasopressin. Lipsmacks ( $P < 0.0001$ ), arching ( $P < 0.0001$ ), tail-lashing ( $P = 0.009$ ), restraining ( $P = 0.015$ ), and approaches to the female ( $P = 0.0002$ ) were all higher during the mirror condition, while tail-twining tended to decline during the mirror condition ( $P = 0.076$ ). Hormones did not vary by experimental treatment, but were correlated with certain behaviors during the presentation of the mirror. While social behaviors changed with mirror exposure, self-directed and mirror-guided behaviors did not, indicating a lack of self-recognition. Use of a mirror was a safe and effective means of investigating mate-guarding behavior in response to a simulated intrusion, with the added benefit of not needing another animal to serve as an intruder; and thus may be of use in providing a laboratory model for natural behavior. Especially, as it eliminates the need for a stimulus animal, it would also be of possible use in investigating responses to a simulated intruder in wild populations of titis and other pitheciines. *Am. J. Primatol.* © 2015 Wiley Periodicals, Inc.

**Key words:** intrasexual aggression; mate-guarding; monogamy; self-recognition

## INTRODUCTION

In nature, titi monkey family groups consist of an adult male, an adult female, and two to four offspring, living together on small territories [Fernandez-Duque et al., 2013; Mason, 1966]. Titi monkeys have been characterized as socially monogamous based on group composition in the wild, preference for a specific partner, distress upon separation from that partner, the ability of the partner to buffer against stress, the presence of biparental care, and mate-guarding [Anzenberger et al., 1986; Cubicciotti & Mason, 1975, 1978; Fernandez-Duque et al., 1997; Mason, 1975; Mason & Mendoza, 1998; Mendoza & Mason, 1986c]. In the field, ritualized encounters with neighbors may occur daily [Mason, 1966, 1968a; Robinson et al., 1987]. Same-sex animals (typically males) engage in mutual chasing with rare but occasional grappling. Although described as aggressive, territorial encounters rarely result in injury or alteration of territorial boundaries. Males will often position themselves

between their mate and potential rivals. Copulation between neighbors has been observed (though infrequently), and males will pursue and restrain mates that move away from them when a rival male is in the vicinity [Mason, 1966, 1968a]. This mate-guarding

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may be part of a strategy for maintenance of exclusivity of the pair.

Laboratory simulations of territorial encounters have been used frequently in the study of socially monogamous rodents (primarily prairie voles, *Microtus ochrogaster*) in order to examine both the neurobiological basis of mate-guarding (although often not in the presence of the mate), and to pharmacologically manipulate this behavior [Aragona et al., 2006; Bales & Carter, 2003; Bowler et al., 2002; Resendez et al., 2012]. In the laboratory as in the field, titi monkeys also behave agonistically toward same-sex strangers [Fernandez-Duque et al., 2000]. In addition to agonistic behaviors, the hypothalamo-pituitary-adrenal (HPA) axis is activated, at least measured by cortisol which is the end-product of the HPA axis. However, this response varies among subjects [Mendoza & Mason, 1986b]. Eight out of ten males will display an increase in cortisol in response to exposure to a same-sex stranger, while only four out of eight females do [Mendoza & Mason, 1986b]. The agonistic reaction to same-sex strangers in male titi monkeys is enhanced when proximity of intruder to the mate decreases [Cubicciotti & Mason, 1978; Fernandez-Duque et al., 2000]. Male titi monkeys have a greater response compared with females when their mates are exposed to strangers [Fernandez-Duque et al., 2000]. These tests have typically been carried out with live stimulus animals, either viewed through a grate or down a long chute in a testing apparatus. Similar live intruder tests have been carried out in other New World monkey species to examine sex differences in reactions to strangers [Wolovich et al., 2010], hormonal responses to intruders [Ross & French, 2011], and effects of group size [Schaffner & French, 1997] among other variables.

For New World monkeys, such as the titi monkey, a mirror image is likely viewed as a same-sex stranger [Anderson & Gallup, 2011b]. If family members, such as the pair-mate, are also present, the stranger could represent a threat to the integrity of the established familial relationships, particularly if the mirror is positioned such that the stranger might represent a territorial intruder. Use of the mirror has the advantage of allowing for testing in the home cage, reducing the number of animals required for the experiment, sparing stimulus animals from a potential stressor, and eliminating the possibility of injury which could occur even if live animals are separated by grates. In laboratory and wild testing, it is also not always possible to have a live conspecific present, thus this represents a solution to what can be a significant logistical challenge. Mirror tests are often used in this way in other taxa such as fish [Braud & Weibel, 1969; Desjardins & Fernald, 2010]. In the present study, we proposed the use of a mirror to simulate a territorial encounter and provide a means to experimentally manipulate mate-guarding

behavior in male titi monkeys; as well as to increase the knowledge base on species which do or do not respond to a mirror image. We hypothesized that arousal and mate-guarding behaviors, such as tail-lashing, arching, and restraint of the pairmate, would increase upon exposure to the mirror. We chose to study males because of their higher reactivity to intruders in previous tests with live intruders [Fernandez-Duque et al., 2000; Mendoza & Mason, 1986b].

We also measured hormonal responses to the mirror encounter to aid in further understanding of the physiology/neurobiology of territoriality. Literature on mating-induced aggression in prairie voles suggests the involvement of oxytocin (OT) and arginine vasopressin (AVP) in mating-induced territoriality [Bales & Carter, 2003; Winslow et al., 1993]. The pituitary peptide AVP acts to augment HPA response peripherally and plays a role in augmenting social aggression centrally [Compaan et al., 1993; DeVries et al., 1997; DeVries & Miller, 1998; Ferris & Delville, 1994; Nelson & Chiavegatto, 2001]. Finally, OT was monitored since its involvement in facilitating affiliation and pair-bonding has been demonstrated in rodents [Cho et al., 1999; Insel & Young, 2001; Young & Wang, 2004] and primates [Smith et al., 2010]. It has also been implicated as a hormone that promotes in-group cohesion at the cost of out-group aggression [Anacker & Beery, 2013; De Dreu et al., 2011; De Dreu & Kret, 2015]. Moreover, it has been suggested that OT can reduce stress responsiveness, acting as an inoculation to physiological reactions to potentially stressful social circumstances [Parker et al., 2005]. Peripheral measurements of OT and AVP may not be reflective of central measures, but may be coordinated in response to social stimuli [Kenkel et al., 2012; Landgraf & Neumann, 2004]. We predicted positive associations of both OT and AVP to reactions to a simulated intruder, based on their strong association with pair-bonding and social displays of aggression.

We also monitored the HPA response in order to evaluate the valence of the mirror stimulus. We hypothesized that cortisol might increase to the extent that the mirror was an aversive stimulus. However, given that cortisol increases in response to a same-sex stranger was not universally displayed even in a live intruder test [Mendoza & Mason, 1986b], to the extent that the mirror test was similar, we would expect to see variation in cortisol responses to a mirror stimulus.

Mirrors have often been used in the context of determining whether or not non-human primates and other animals can recognize themselves, i.e. demonstrate theory of mind [Anderson & Gallup, 2011b; Gallup, 1970]. Only great apes (not even lesser apes: [Suddendorf & Collier-Baker, 2009]) show undisputed self-recognition [Anderson & Gallup, 2011b], although there is some controversy regarding rhesus

monkeys [Anderson & Gallup, 2011a; Rajala et al., 2010]. Other New World monkeys tested, for example capuchins, do not show evidence of self-recognition in a mirror in several studies [Anderson & Roeder, 1989; Roma et al., 2007], although there is some evidence for differential responses to mirrors and strangers in at least one study [de Waal et al., 2005]. The classic method of determining self-recognition starts with spontaneous observation of mirror-guided behaviors (for instance, using a mirror to look at areas of the body that are not normally visible). Researchers have also used increased self-directed behavior (ex. increased self-grooming or touching of parts of the body) as early indicators of self-recognition. These behaviors should increase over time, as social responses to the mirror decrease. These observations are then followed by a formal mark test (marking a non-visible part of the body, such as the forehead, when the animal is unaware, and then observing mirror behavior) [Gallup, 1970]. Although testing self-recognition was not a goal of this study, we also coded the videotapes for mirror-guided and self-directed behaviors, with the prediction that titi monkeys would not show signs of self-recognition in the mirror.

## METHODS

### Subjects and Housing

Subjects for this study were eight paired male coppery titi monkeys (*Callicebus cupreus*), with a mean  $\pm$  (SEM)  $8.5 \pm 1.3$  years of age at the beginning of the project. All subjects were housed with their mates and any immature offspring (see Table I for detailed Subject information). Family groups were housed in  $1.2 \text{ m} \times 1.2 \text{ m} \times 2.1 \text{ m}$  stainless steel cages with four horizontal perches extending the width of the cage at varying heights; there was also a small U-shaped perch ( $15.9 \text{ cm} \times 15.9 \text{ cm}$ ) on the door of the cage. Animals were fed twice a day with a diet consisting of New World monkey chow, rice cereal, carrots, apples, raisins, and bananas. A more detailed account of husbandry is available in Tardif et al. [2006] and Mendoza & Mason [1986a]. Water

was available ad lib. This study complied with IACUC protocols, legal requirements of the United States, and the policies of the American Society of Primatologists on ethical treatment of animals.

### Mirror Exposure and Behavioral Assessment

A mirror (approximately  $36 \times 22 \text{ cm}$ , on top of a movable cart  $82.6 \text{ cm}$  in height) was wheeled in front of each animal's homecage to signal the start of the experimental session. The mirror was placed directly in front of the cage door so that an animal sitting on the small door perch would see the full image of itself sitting approximately  $16.5 \text{ cm}$  away. It is also possible that the male would see a reflection of its mate and/or offspring in the mirror as well. Each session consisted of a 5-min exposure to the cart and mirror in one of two orientations: (1) Mirror condition with the reflective surface facing toward the subject's cage or (2) Control condition with non-reflective back of the mirror facing the subject's cage. Each subject was tested twice per week, once in each condition with test days separated by at least one non-test day and the order of testing counter-balanced. Testing continued for 5 weeks. Subjects were filmed during the test and scored later. Blood samples were taken during both conditions on weeks 2 and 4 of the experiment. Therefore, the total experiment consisted of 10 exposures (five Mirror, five Control) and four blood samples (two Mirror, two Control). Social behaviors, as well as location in the cage, were scored using Behavior Tracker 1.5 ([www.behaviortracker.com](http://www.behaviortracker.com)). Behavioral definitions are detailed in Table II. We also scored mirror-guided and self-directed behaviors to examine for self-recognition in the mirror. Offspring were left in the cage during testing.

### Blood Collection and Processing

Four blood samples (1 ml) were collected from each subject via femoral venipuncture. Subjects were unanesthetized, and blood was collected using 1-cc syringes pretreated with heparin. Subjects were

**TABLE I. Subject Information Including Age, Pairing Length, Number of Offspring Living With the Pair, and Oxytocin Levels in the Control Condition (Averaged From Both Samples)**

Male ID	Age (years)	Pairing length (years)	Number of offspring in cage	Oxytocin (pg/ml) in control condition
29775	14.8	3.2	0	$770.65 \pm 21.44$
31716	11.9	9.0	2	$651.79 \pm 51.38$
32878	10.1	1.0	0	1,540.71 (second sample not available)
34438	8.5	3.2	0	$657.89 \pm 62.19$
34531	8.2	3.9	2	$986.07 \pm 56.15$
36150	6.4	0.7	1	$890.21 \pm 32.22$
36187	6.1	1.2	1	$766.08 \pm 51.68$
38644	3.2	0.9	1	$671.43 \pm 186.55$

TABLE II. Stimulus Exposure Ethogram

Behavior	Definition
<b>Social behaviors</b>	
Contact	Passive physical contact between male and pair-mate that does not include tail-twining.
Proximity	Male comes within arm's reach of female.
Male groom	Male combs through fur of female.
Female groom	Female combs through fur of male.
Tail-twining	Male sitting side by side with female, with tails wrapped around each other for at least one rotation.
Male approach	Male moves toward female and establishes contact.
Male leave	Male breaks contact with female.
Lipsmack	Male repeatedly and rapidly opens and closes mouth.
Chest rub	Male moves chest with pressure and friction against a perch or surface; may also press downward on chest with hands or arms.
Tail-lashing	Male repetitively swings whole tail from side to side (area greater than 40°, usually sign of agitation).
Aggression	Male displays aggression to female.
Mount/sex	Male mounts female or attempts/succeeds to copulate.
Restraining	Male forcibly restrains female by placing hands on her shoulders.
Arching	Male arches back (as in a frightened cat), the subject may also have his arms and trunk lifted off the perch.
<b>Location</b>	
Top perch	Male located on highest perch.
Off camera	Male not visible in viewing area.
Small door perch	Male located on door perch closest to mirror.
<b>Mirror-guided behaviors</b>	
	Using the mirror to adjust movements of the body.
	Using the mirror to view body parts not normally visible.
<b>Self-directed behaviors</b>	
	Using the mirror to look at environment not normally visible.
	Looking behind the mirror.
	Self-grooming.
	Touches to the body.

captured in a transport cage, which they had all been trained to voluntarily enter upon demand. Animals were then hand captured and manually restrained for sample collection. Average time of blood collection from entrance to the cage was  $180.23 \pm 13.16$  sec, while average time from removing the monkey from the transport box until blood draw was  $98.55 \pm 12.68$  sec. There was no difference between treatment conditions in either measure ( $P > 0.05$ ). During week 2 of testing, blood samples were collected 20 min following first exposure to the mirror or the control. These samples were subsequently assayed for plasma cortisol concentrations. During week 4, blood samples were collected 5 min after initial exposure (immediately following behavioral testing) in order to capture responses for the more rapidly reactive peptide hormones AVP and OT. Following blood collection, samples were immediately placed on ice, centrifuged at 3,000 rpm at 4°C, and the plasma extracted and stored at -80°C until assay.

Plasma cortisol concentrations were measured using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA) previously validated for use in titi monkeys [Hoffman et al., 1995]. Samples were diluted 1:4 in PBS-

gel and all samples were run in a single assay. Intra-assay coefficient of variation was 2.45%. OT and AVP were analyzed by enzyme immunoassay (Enzo Life Sciences, Farmingdale, NY) validated for titi monkeys [Bales et al., 2005], with samples diluted at 1:6. Intra-assay coefficients of variation were 3.72% for OT and 4.49% for AVP. Although we originally collected the 5-min samples for AVP and OT analysis and the 20-min sample for cortisol, we decided to assay AVP and OT at both time points. This allowed us to examine effects of time elapsed since the stimulus presentation on these two peptides, in order to establish a time course for future studies.

### Data Analysis

Data were analyzed by general linear mixed model ANOVA [Littell et al., 1996] in SAS 9.3 (SAS Institute, Cary, NC). Models included condition (mirror or no mirror), week, a condition by week interaction, and male identity (ID) as a random factor, which accounts for the repeated measures design of the experiment. Due to very low levels of mirror-guided and self-directed behaviors, these were compared by condition but not by week. Residuals were

checked for non-normality and transformed as necessary. Spearman correlations for hormone-behavior relationships were carried out on a limited number of behavioral variables (arching, tail-lashing, tail-twining, and approaches to the female vs. OT, AVP, and cortisol). We compared only Control condition behavioral variables to Control condition hormonal variables, and Mirror condition behavior to Mirror condition hormones. We chose to use these non-parametric correlations because there were a number of zeros for certain behaviors particularly in the Control condition, causing the correlations to be more driven by a few data points. Both time points were included for correlations. Because we performed correlations on only a limited number of variables with strong a priori hypotheses regarding correlations, we did not use post-hoc corrections for multiple comparisons. All tests were two-tailed and significance was set at  $P < 0.05$ .

## RESULTS

### Behavioral Responses to Mirror Exposure

There was no effect of week in any of the behavioral models ( $P > 0.05$  for all tests), and the week by condition interaction is reported below only when significant ( $P < 0.05$ ). Males spent more time on the small door perch (and therefore closest to the mirror) during the Mirror condition (condition,  $F_{1,62} = 8.60$ ,  $P = 0.006$ ). During the Mirror condition, the mean duration of sitting on the door perch was  $36.3 \pm 0.68$  times during the 5 min observation, while in the Control condition, it was  $12.67 \pm 0.33$  times. Time spent on the highest perch (farthest from the mirror) and time off camera were not altered by experimental condition.

As predicted, agonistic displays were more frequent during the Mirror condition than the Control condition. Tail-lashing occurred at a significantly higher rate during the Mirror condition (treatment,  $F_{1,62} = 7.33$ ,  $P = 0.009$ ) (Fig. 1). Similarly, arching occurred at a significantly higher rate during the Mirror condition (treatment,  $F_{1,62} = 19.36$ ,  $P < 0.0001$ ) (Fig. 1). Behavior toward the female pair-mate was also altered by the presence of the mirror. Lipsmacking toward the mate occurred at a significantly higher rate during the Mirror condition (treatment,  $F_{1,62} = 22.94$ ,  $P < 0.0001$ ) (Fig. 1). There was a condition by week interaction ( $F_{4,62} = 2.71$ ,  $P = 0.038$ ), with lipsmacking increasing in the Mirror condition and decreasing in the Control condition. Males restrained their mate more often in the Mirror condition ( $F_{1,62} = 6.24$ ,  $P = 0.015$ ) (Fig. 1). Although frequency or duration of contact with the mate was not affected by condition, tail-twining tended to be lower during the Mirror condition (treatment,  $F_{1,62} = 3.25$ ,  $P = 0.076$ ) (Fig. 1). Approaches to the female were significantly higher in the Mirror condition

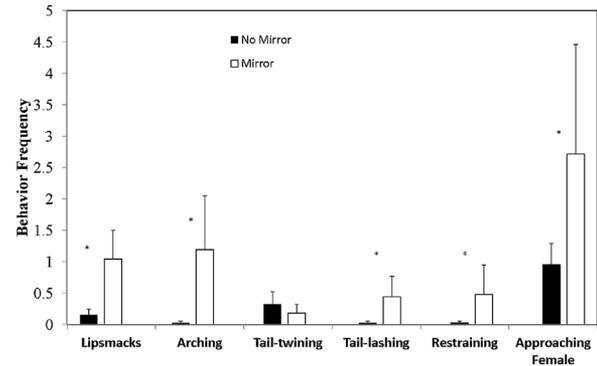


Fig. 1. Frequency of male behaviors (mean  $\pm$  standard error) for the different experimental conditions (mirror or back of mirror). \* $P < 0.05$ .

( $F_{1,62} = 5.76$ ,  $P = 0.019$ ), while leaves did not differ significantly by treatment (Fig. 1).

In all behavioral measures, there was considerable variability among males ( $P < 0.01$  for the random effect of ID). There were no differences between conditions in time spent grooming or in male chest-rubs. Aggression and mount/sex occurred too infrequently for analysis.

### Endocrine Responses to Mirror Exposure

Cortisol was not significantly higher in response to the Mirror condition when compared with the Control condition ( $F_{1,13} = 0.03$ ,  $P = 0.865$ ). Cortisol levels were  $35.36 \pm 5.26$   $\mu\text{g/dl}$  (mean  $\pm$  SEM) in the Control condition and  $38.36 \pm 5.81$   $\mu\text{g/dl}$  in the Mirror condition. There was no significant change in OT in response to condition ( $F_{1,25} = 0.25$ ,  $P = 0.620$ ), time to blood collection ( $F_{1,25} = 0.35$ ,  $P = 0.560$ ), or a condition by time to blood collection interaction ( $F_{1,25} = 1.72$ ,  $P = 0.201$ ). OT levels were  $821.93 \pm 63.46$   $\text{pg/ml}$  in the Control condition and  $881.5 \pm 85.65$   $\text{pg/ml}$  in the Mirror condition. There was no significant change in AVP in response to condition ( $F_{1,25} = 0.14$ ,  $P = 0.710$ ) or the condition by time interaction term ( $F_{1,25} = 0.92$ ,  $P = 0.347$ ); however, there was a significant effect of time post-mirror exposure ( $F_{1,25} = 8.06$ ,  $P = 0.009$ ), with AVP higher at 20 min post-exposure than at 5 min. AVP levels were  $302.23 \pm 23.57$   $\text{pg/ml}$  in the Control condition at 5 min and  $507.83 \text{ pg/ml} \pm 88.48$  in the Control condition at 20 min. In the Mirror condition, AVP levels were  $326.45 \pm 36.18$   $\text{pg/ml}$  at 5 min and  $461.28 \pm 80.95$   $\text{pg/ml}$  at 20 min. In all hormonal measures, there was considerable variability among males ( $P < 0.01$  for the random effect of ID).

### Mirror-Guided and Self-Directed Behaviors

There were no significant effects of condition on mirror-guided or self-directed behaviors (Table III), except for number of touches to the face ( $F_{1,71} = 4.01$ ,

**TABLE III. Mirror-Guided and Self-Directed Behaviors (Mean  $\pm$  Standard Errors)**

Behavior	Control	Mirror
Look behind the mirror	0.075 $\pm$ 0.042	0.075 $\pm$ 0.055
Double-take	0	0.05 $\pm$ 0.035
Examine parts of body not normally visible	0	0.075 $\pm$ 0.055
Use mirror to adjust movements of the body	0	0
Use mirror as a tool to look at environment	0	0
Touch head	0.025 $\pm$ 0.025	0.075 $\pm$ 0.055
Touch face	0.275 $\pm$ 0.101	0.05 $\pm$ 0.035
Touch chest	0	0.075 $\pm$ 0.075
Touch arm	0	0.1 $\pm$ 0.06
Touch hand	0.025 $\pm$ 0.025	0.05 $\pm$ 0.025
Touch stomach	0.025 $\pm$ 0.025	0.05 $\pm$ 0.05
Touch body	0.075 $\pm$ 0.042	0.15 $\pm$ 0.127
Touch foot	0.05 $\pm$ 0.035	0.025 $\pm$ 0.025
Touch tail	0.025 $\pm$ 0.025	0
Touch back	0.125 $\pm$ 0.082	0.1 $\pm$ 0.078
All touches to body parts	0.625 $\pm$ 0.159	0.65 $\pm$ 0.296
Self-groom	0.025 $\pm$ 0.025	0

$P=0.049$ ). However, touches to the face were actually higher in the Control condition (Table III).

### Hormone-Behavior Relationships

In the Control condition, hormonal measures were not correlated with any behavioral variables examined (arching, tail-lashing, tail-twining, and approaches to the female). In the Mirror condition, AVP was positively correlated with tail-lashing ( $r_{14}=0.562$ ,  $P=0.036$ ), and there were non-significant trends for positive correlations with arching ( $r_{14}=0.518$ ,  $P=0.057$ ) and tail-twining ( $r_{14}=0.530$ ,  $P=0.051$ ) (Fig. 2). OT was positively correlated with tail-lashing ( $r_{14}=0.665$ ,  $P=0.009$ ) and arching ( $r_{14}=0.626$ ,  $P=0.017$ ), and there was a

trend for a positive correlation with approaches to the female ( $r_{14}=0.504$ ,  $P=0.066$ ) (Fig. 3).

### DISCUSSION

In monogamous species, behaviors such as affiliation and mate-guarding, can help maintain the pair-bond and support the reproductive success of the pair [Anzenberger, 1988; Fuentes, 1999; Kleiman, 1977; Robinson et al., 1987]. In another socially monogamous species, owl monkeys (*Aotus*), a recent field study showed that replacement of pair-mates by intruders results in negative effects on the reproductive success of both partners; maintenance of the pair-bond may, therefore, be adaptive in many cases [Fernandez-Duque & Huck, 2013]. The present

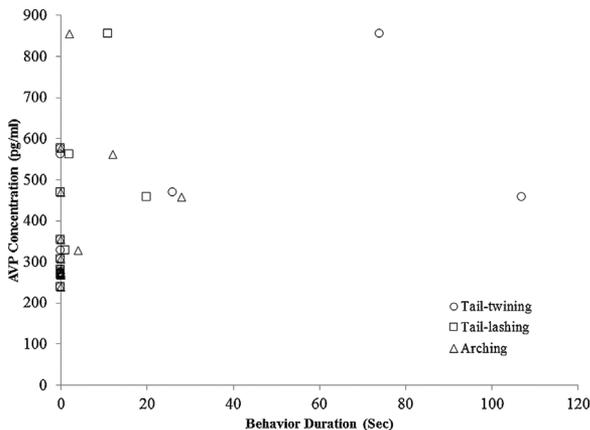


Fig. 2. Correlation of male AVP concentration (pg/ml) to duration of tail-twining, tail-lashing, and arching in mirror condition only.

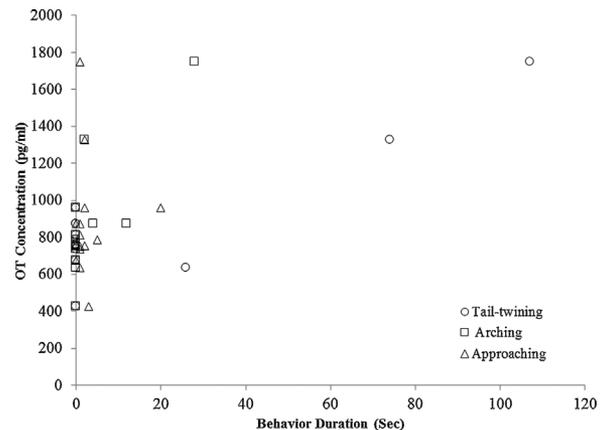


Fig. 3. Correlations of male OT concentration (pg/ml) to durations of arching, approaching, and tail-twining in the mirror condition only.

study shows that male titi monkeys will react to a mirror image as though an intruder is present. Agonistic and arousal behaviors, such as arching, tail-lashing, and time spent near stimulus, significantly increased during the Mirror condition, which indicates an agitated state and interest in the “other animal”. Males also increased active interactions with their pair-mates, including lip-smacking, restraint, and approaches.

In the wild, titi monkeys show a large degree of coordinated behavior [Mason, 1966], including spending large amounts of time in proximity or contact to the mate [Fernandez-Duque et al., 2013; Mason, 1968b], grooming the mate [Fernandez-Duque et al., 2013; Kinzey & Wright, 1982], engaging in duets [Robinson et al., 1987], sleeping in a huddle with twined tails [Souza-Alves et al., 2011], and performing biparental infant care [Kinzey et al., 1977; Mason, 1966]. They also show territorial behaviors as described in the Introduction section, including chasing of intruders and restraint of mates [Mason, 1966, 1968a; Robinson et al., 1987]. In our study, titi monkey males confronted by a mirror, thus, significantly increased the same behaviors associated with intruders in wild titi monkeys.

One limitation of this study was the lack of a live intruder control condition, which would have allowed a direct comparison between these two methods [de Waal et al., 2005]. However, there is an extensive literature allowing us to quantitatively compare our results to those from live intruder paradigms in captive titi monkeys [Cubiciotti & Mason, 1978; Fernandez-Duque et al., 1997, 2000; Mendoza & Mason, 1986b; Menzel, 1986, 1993]. When exposed to a live male stranger, male approaches to the mate increased by 2.57 times in frequency in the Mendoza and Mason paper [Mendoza & Mason, 1986b]; approaches to the mate increased by 2.87 times in this study when males were confronted with a mirror. Approaches to the male stranger (2.52 times higher than control) were also similar to approaches to a mirror, defined here as being located on the small door perch (2.03 times higher than control). With a live intruder, displays (lip-smacking and back-arching) increased by 54 times in frequency, whereas in this study back-arching increased by 47.71 times. Thus, behavior toward a simulated intruder appears quantitatively similar to that toward a live intruder.

OT did not vary consistently between the experimental conditions. However, OT was correlated with certain behaviors which promote maintenance of the pair-bond. During the mirror condition, OT was positively correlated with tail-lashing, arching, and tended to correlate with approaches to female. These correlations were often driven by a few data points from males that had both high OT and high behavioral arousal. As in rodents [Carter & Keverne, 2002; Insel & Young, 2001; Young et al., 2008; Young & Wang, 2004], these data suggest that

animals that have more active OT systems also engage in more active expressions of affiliation toward the mate, and more mate-guarding in the presence of a potential challenge to the bond. In contrast to our expectations, AVP did not respond differentially to treatment conditions and did not correlate with expression of agonistic behavior toward the mirror or mate-guarding. AVP was elevated 20 min post exposure, suggesting some hormonal response to the procedure, although levels did not differ between animals exposed to the front or the back of the mirror, possibly because the pair-mate was present to buffer a stress reaction [Ragen et al., 2013]; but this suggests that animals were mildly responsive to the novelty of the procedure as a generalized stressor. As the only previous studies on titi monkey plasma OT and AVP in the literature were focused on the effects of opioid manipulations [Ragen et al., 2013, 2015], this paper presents the first report of OT and AVP levels in titi monkeys in the context of a behavioral manipulation. We also did not find any effect of the mirror manipulation on cortisol. However, the live intruder study [Mendoza & Mason, 1986b] also found variation between males. It is possible that variation in cortisol levels, as well as other hormonal levels, reflects differences in the strength of the pair-bond.

Were male titi monkeys responding to a simulated intruder, or to what they recognized as their own reflection? While we did not do a conclusive test of self-recognition, we examined behavior for any potential signs of self-awareness as examined in other studies of mirror behavior [Bard et al., 2006; de Waal et al., 2005], and did not find them. Gallup [1970] considered lowered social responses, and increased self-directed responses during mirror exposure, to be signals of self-recognition. In contrast, here we found increasing social responses to the mate across time during mirror exposure (and no change across time in other social responses), with self-directed behaviors at too low of a frequency to even analyze across time. These results are not surprising given that self-recognition has only been shown conclusively in the great apes. However, this does add another New World monkey species to the list of those that have been examined. This analysis also lends support to the assertion that titi monkey males were treating the mirror as a novel conspecific, not as their own reflection. While mirror-guided and self-directed behaviors did not differ between conditions, arousal behaviors indicative of a challenge (arching, tail-lashing), affiliative behaviors (approaches to the female, lip-smacking), and mate-guarding behaviors (restraining the female) increased in the mirror condition. We would, therefore, conclude that the mirror was viewed as a territorial intruder and not as the subject himself.

While we have discussed the use of this test primarily in the context of social monogamy, it could

theoretically be used to answer many questions, in different species of Pithecines, regarding social reactions to simulated same-sex conspecifics. For instance, wild male *Chiropotes* display a ritualized “lining-up” behavior in which they engage in affiliative contact with other in-group males [Garber & Kowalewski, 2013; Veiga & Ferrari, 2013]. Reactions to a “strange male” in the mirror may be interesting in this species, particularly depending on whether the test male is with his group-mates or not. In addition, a mirror stimulus could be relatively easily placed and used in the wild, in a manner similar to other psychological testing [Martin & Santos, 2013] or play-back experiments [Cheney & Seyfarth, 1999].

In summary, the present study confirms the hypothesis that male titi monkeys react behaviorally to a mirror image similarly to their reaction to a strange male. Our previous research has shown that male titis respond to the actual presence of a stranger with the full panoply of behavioral changes reported here [Fernandez-Duque et al., 2000; Mendoza & Mason, 1986a]. While the simulated territorial encounter did not result in changes in cortisol, OT, or AVP, the correlation of OT and AVP to specific behaviors, such as arching, lip smacking, and tail twining, suggests that one avenue for future research might be in ways that individual differences in the hormones may reflect individual differences in the pair-bond. Finally, these results suggest that a simulated territorial encounter in the laboratory can be useful to address questions that are of interest to socioecologists, but difficult to answer in wild populations.

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