Female social behaviour during three male introductions in captive groups of rhesus macaques

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ABSTRACT

Introductions of new males into captive primate groups are often necessary to prevent inbreeding, but also bear high social risks. To minimize these risks, it is crucial to understand the social behaviour accompanying male introductions. While the behaviour of new males is generally understood, information on resident female behaviour during introductions is lacking. We studied female behaviour towards the new male during introductions of three adult male rhesus macaques *(Macaca mulatta)*—each into a different captive group. All three males were successfully introduced; respectively 100%, 92%, and 83% of the females tolerated the male as a group-member at the end of the introductions. Older females started tolerating the male significantly faster than younger females, while no additional effect of female dominance rank, fertility, or the number of female coalitionary partners on timing of tolerance was found. During the course of the integration, female aggression and submission towards the male, and male mating access decreased, while female affiliation towards the male increased. The increase of female tolerance and the changes in social behaviour were similar between the introductions, indicating a general pattern in female behaviour, although some variation in effect size and significance level was observed. Based on these results, we suggest that low female submission levels towards an introduced male may constitute a criterion to assess the risk of leaving the male in the group full-time. Moreover, low female aggression levels at the end of the introduction may signal long-term group stability. Overall, we conclude that female behaviour can provide valuable information about the male introduction process and should not be overlooked.

1. Introduction

Nowadays, naturalistic group housing is the preferred way to house captive social animals. Naturalistic group housing not only concerns a natural, or close to natural group composition, but also mimicking of group dynamics observed in the wild, such as migration patterns. In the wild, migration promotes gene flow between groups and prevents inbreeding (Krause and Ruxton, 2002; Moore and Ali, 1984). Similarly, in captivity inbreeding is often prevented by introducing unfamiliar animals into existing social groups. The introduction of unfamiliar animals is thus necessary for captive management, but also bears risks. The social risks of immigration found in wild animals may also be present during captive introductions mimicking the wild immigration process.

The risk of immigration of unfamiliar animals into a group is especially well reported in primates. Migration in primates is often male-biased (Greenwood, 1980). Primate males face the challenge of immigrating into a social group, comprised of a cohesive core of related

resident females, and obtaining a new position in the social network. This challenge is especially prominent in species that live in multi-male multi-female groups. The entrance of new males in such groups is associated with high levels of aggression, in both captive and wild situations (captivity: Bernstein et al., 1977; Rose et al., 1972, wild: Alberts et al., 1992; Bercovitch, 1997; Marty et al., 2017; Teichroeb et al., 2011; Zhao, 1994). Additionally, there is risk of injuries for adults of both sexes (wild: Marty et al., 2017; Packer, 1979; Pereira, 1983; Zhao, 1994, free-ranging: Lindburg, 1969), high stress levels (wild: Alberts et al., 1992; Marty et al., 2017) and low immune resistance (wild: Alberts et al., 1992). Moreover, new males can be infanticidal, posing a threat to resident females' young (captivity : Zaunmair et al., 2015, wild: Borries, 1997; Hrdy, 1979; Pereira and Weiss, 1991; Sterck and Korstjens, 2000; van Belle et al., 2010). Males can vary in their behaviour when entering a group; some males are aggressive while others may be more unobtrusive (wild: Marty et al., 2016; Van Noordwijk and Van Schaik, 2000, 1985). The male's behavioural

strategy may influence his success in establishing a position in the new group. A male may not be accepted by resident group members, and thereby not succeed in entering the group (captivity: Bernstein et al., 1977, free-ranging: Vessey, 1971, wild: Neville, 1968; Packer, 1979; Packer and Pusey, 1979; Van Noordwijk and Van Schaik, 1985; Yamada, 1971, 1963). These risks associated with the entrance unfamiliar primate males into existing social groups are well acknowledged, yet the behavioural process accompanying integration (i.e. establishing a social position) is understudied.

Our current knowledge on social behaviour during integration mainly derives from relatively old, often descriptive studies of both wild and captive primates, which focus on the new male's behaviour. These studies report that initial high levels of aggression decrease over time (captivity: Bernstein et al., 1977; Rose et al., 1972). After a brief period of aggression, new males can engage in grooming with resident females (captivity: Bernstein et al., 1977). Moreover, high levels of submissive behaviour commonly accompany interactions between unfamiliar animals (captivity: Bernstein and Mason, 1963; Rose et al., 1972). Overall, these studies provide general understanding of the behaviour of new males. However, the role of resident females during male entrance has not received much attention, although their tolerance of the new male is probably crucial for successful integration (wild: Yamada, 1971). The few studies addressing female behaviour during the entrance of a new male show contrasting results. Some females may not tolerate a new male and refrain from associating, while others respond with aggression (captivity: Bernstein et al., 1977; Kawai, 1960; Rose et al., 1972). Females may employ aggression to prevent new males from entering a group through the formation of coalitions (wild: Packer and Pusey, 1979). Female aggression towards new males may be absent in successful introductions (captivity: Zaunmair et al., 2015), but also when female aggression is present, it may quickly be replaced by affiliation (captivity: Bernstein et al., 1977). Moreover, females may engage in high levels of mating immediately after male entrance into a group, since females prefer to mate with novel males (captivity: Bernstein and Mason, 1963; Rose et al., 1972, free-ranging: Manson, 1995, 1994, wild: Packer, 1979), and submission levels are high (captivity: Rose et al., 1972). However, no quantitative data on female behaviour towards new males are available, although their behaviour is probably directly linked to introduction success. In addition, female behaviour may differ depending on their individual characteristics. Younger females compared to older ones may be more afraid of the new male (Bernstein and Ehardt, 1985). Furthermore, high ranking females may associate more often with the new male (Kawai, 1960). In addition, females whose fertility occurs sooner after the start of the introduction may tolerate the new male sooner, since fertile females associate more often with unfamiliar males than non-fertile females (captivity: Zaunmair et al., 2015). Lastly, females forming larger coalitions may tolerate the male later, since their coalitions are stronger and may thus be better able to prevent male entrance. Moreover, females disliking the new male may be more likely to form coalitions. Altogether, it is crucial to increase our quantitative understanding of resident female behaviour during male introductions for the improvement of introduction management procedures.

This study has two aims: (1) to identify the female characteristics that influence female behaviour towards new males and (2) to provide a general understanding of female social behaviour during male introductions. To this end, female behaviour towards the new male was studied during introductions of three adult male rhesus macaques *(Macaca mulatta)*—each into a different captive social group consisting of adult females and their immature offspring. Though wild rhesus macaques generally live in multi-male multi-female groups, there is often only a single male present in captive groups. Creating single-male, multi-female groups prevents male-male competition, which bears high risks in a confined enclosure. In our study, single-male, multi-female groups were created, and natural migration patterns were mimicked.

During the introductions, we expect variance in female tolerance of the new male, possibly depending on individual female characteristics, namely her age, dominance rank, fertility, and on the number of her female coalitionary partners. In addition, we expect that female social behaviour towards the new male changes during the introductions, namely that initial high levels of female aggression, submission and mating towards the new male decrease and initial low levels of affiliation increase. Finally, we studied the new males' behaviour at the start (i.e. first five hours) of their introductions, since this may explain possible differences in female behaviour. This part of our study is by necessity explorative due to the limited number of introductions.

2. Methods

2.1. Subjects and housing

Subjects were 41 female rhesus macaques, ranging in age from 3 to 19 years, living in three different social groups (the Liby group, the Marieke group, and the Clio group). The groups consisted of 12 to 16 adult females ($\geq$ 3 years of age) and their non-adult offspring (Table 1–3 in Online Resource 1). Female offspring remain in the group during their whole life. Male offspring are removed around 4 years of age, mimicking natural migration processes.

The groups were housed at the Biomedical Primate Research Centre (BPRC) in Rijswijk, the Netherlands. The enclosures contained multiple elevated beams and environmental enrichment items (Vernes and Louwerse, 2010). The inside enclosure measured 72 m^2 and was 2.85 m high, the outside enclosure measured 208 m^2 and was 3.1 m high. The three compartments of the outside enclosure were separated by wire mesh. Concrete walls separated inside compartments one, two, and three. Inside compartment four was separated from the compartments two and three by wire mesh (Fig. 1 in Online Resource 1). Outside introductory events, the resident group had access to inside compartments one and two and all three outside compartments and the new male was housed in inside compartments three and four. Animals received standard monkey chow [Ssniff©] in the morning and fresh fruit, vegetables or bread in the afternoon. Water was available *ad libitum*

2.2. Introduction process

Three adult males were introduced during this study—each into a different social group. All three introductions took place during the breeding season and will hereafter be referred to as introduction A (Liby group), introduction B (Marieke group), and introduction C (Clio group). The introductions were part of the regular management procedures at BPRC and followed the BPRC introduction guidelines. An experienced animal caretaker carried out the introductions, deciding on the duration of the different phases of an introduction by estimating the risks of severe attacks from the females to the new male and vice versa, based on personal knowledge and experience. Before the introduction, the residents and the new male were familiarized with each other, by allowing interaction through wire mesh. The male was then introduced to the group. During the introduction, contact possibilities between the resident females and the new male, and time of contact were progressively increased. At first, the animals only had access to the outside enclosure during introductory events. After four to seven days of contact, the animals were given additional access to the inside enclosure during introductory events. The duration of these introduction events began at 1–1.5 h on the first day and was gradually increased to approximately 7 h per day. The exact timing typically differed between introductions. Ultimately, the male was allowed to remain in the group full-time: this happened after 12, 24, and 20 days with physical contact for introductions A–C, respectively.

The husbandry process of introducing a new male needs to be distinguished from the male's integration process. The *introduction* of an animal is a husbandry process. An introduction is considered successful when the new male is allowed to remain in the group full-time for at

least 4 weeks (Brent et al., 1997). This criterion is based on introductions of chimpanzees (*Pan troglodytes*), with the aim of composing long-term stable groups, comparable to our study. *Integration*, in turn, concerns the process of establishing a social position in the group, and can be studied by looking at an animal's behaviour. While the introduction process is typical for captivity, the integration process takes place both in captive and wild groups. Whether a male successfully integrates into a group is based on female tolerance of the new male (see *Measures* below, based on: Bashaw et al., 2010; Meder, 1990).

2.3. Behavioural observations

Data were collected between December 2014 and May 2015. Continuous focal observations of the new male and his female interaction partners were carried out during the study. We scored the social interactions among the new male and the females whenever the male was visible to the observer. All aggressive, unprovoked (i.e. not in response to aggression) submissive and affiliative behaviour from the females towards the male and *vice versa* was recorded. Furthermore, we scored all approaches from and towards the male, whether these were accepted (i.e. remain within 1 m for at least 3 s) or rejected (Kempes et al., 2008), and all mating events. Finally, submission, i.e. bared teeth and give ground behaviour between all group members was scored *ad libitum*. For a detailed description of the recorded behaviours, see the ethogram in Online Resource 2.

Behavioural observations were conducted whenever the new males spent time with the group, on workdays between 9:00 h and 12:00 h, and between 13:00 h pm and 16:00 h. Observations started at the first day of physical contact and ended 2 (introductions A and C) to 4.5 (introduction B) weeks after the completion of the introduction (i.e. the male was allowed to remain in the group full time). In total, 48 (introduction A), 95 (introduction B), and 66 (introduction C) hours of observational data were collected, of which the male was visible for 24 (introduction A), 44 (introduction B), and 33 (introduction C) hours. A different observer observed each introduction. All observers were trained until they reached agreement with the first author. The location of the observations (50% inside and 50% outside) followed a semi-random schedule when the animals had inside and outside access.

2.4. Fertility scoring

Female sex skin coloration was used as a measure for fertility during introductions A and C. The sex skin coloration of each female was scored once every observation day. Skin coloration was measured at three locations on the face and one location on the hindquarters (cf. Dubuc et al., 2009), using Pantone© colour scales. Fertility scores ranging from 1 to 12 were assigned to the Pantone C and PC colours 1767, 1777, 1787, Red 031, 1797, and 1807. The lightest colour received score 1 and the darkest colour received score 12. Female sex skin coloration was assigned the same score as the Pantone colour it resembled most closely. The comparisons were made from a distance of approximately 5 m. Visual comparisons are known to be equally reliable as the digital analysis of red, green, and blue reflectance values (Dubuc et al., 2009). All animals in a group were scored in the same area of the enclosure and under similar artificial lighting conditions. The observers were trained until they reached agreement with the first author in real life observations. Moreover, more than 80% agreement was reached in a picture test.

Monthly peaks in female sex skin coloration were only visible on the hindquarters and not on the face. Two females in introduction C showed no variation in sex skin coloration.

2.5. Measures

Female tolerance of the new male occurred when he gained social access to her (based on: Bashaw et al., 2010; Meder, 1990). Female tolerance was considered to have been granted by a female when at least one of the following is true: 1) she accepted at least 3 of the last 4 approaches by the male; 2) she approached the male at least twice non-aggressively; 3) she had been together (i.e. within 1 m for at least 3 s) with the male at least twice outside the context of sexual encounters; or 4) she groomed, or was groomed by, the male at least once. For the analysis, intolerant females (N = 3) were considered to have tolerated the new male on the last observation day + 1 day, based on the assumption that they would have tolerated the male later. In any event, the results were hardly affected when these three individuals were excluded from the analysis.

The female characteristics used in this study concerned four different individual measures. The first measure is female age on the first day the male was introduced. The second measure is female dominance rank in the social hierarchy which is based on bared teeth and give ground (Altmann, 1962; de Vries, 1998). The female dominance hierarchy was significantly linear in all three groups (Liby group: h' = 0.96; Marieke group: h' = 0.85; Clio group: h' = 0.80, all p ≤ 0.002; h' is Landau's linearity index corrected for the number of unknown relationships (de Vries, 1995). The dominance rank was set on a scale with the most dominant female ranking 1. The third measure is the first day a female achieved the highest fertility score on her hindquarters. We considered this to match her first peak in fertility during the introduction. Females not showing any variation in sex skin coloration (N = 2) were considered to have their peak at the last observation day + 1 day. In any event, the results were hardly affected when these two individuals were excluded from the analysis. The fourth individual measure is the number of female coalitionary partners that each female has; this was determined by the total number of different females aged ≥ 3 years that supported the subject in conflicts with the new male. A female supported another female when she joined an already present conflict by displaying aggressive behaviour towards the male or his supporters.

The number of successful copulations between the females and the new male, regardless of female identity, was used as a measure of male mating access. Since female collaboration is required for mating, this is not only determined by the male. Consistent with this view, forced mating by the male was rare (5 out of 1661 copulations) and females could successfully reject male mating attempts (90 out of 1223 male-initiated mating attempts) (cf. Overduin-De Vries et al., 2012).

2.6. Data analysis

To identify the new male's behavioural strategy, rates per hour of his aggression, submission, affiliation, and mating were calculated for the first 5 h a male had physical access to the females (covering the first 4 (introduction A), 5 (introduction B), and 3 (introduction C)) days of the introduction. We choose an equal period of time rather than an equal number of days so that all males had equal opportunities to interact with the females. A male's initial attitude towards the females was typically observable during this time frame. Note that our sample size of three males is too small to allow for a meaningful statistical analysis of male behaviour.

The focus of this study was on female social behaviour, which was analysed using the following approach. First, the day of tolerance was determined for each female. Next, general linear models, each including separate slopes for the three introductions, were fitted to test the effect of (1) female age, (2) the inverse of female age (1/age; this transformed variable is used to test for a non-linear (i.e. negative exponential) relationship between age and day of tolerance), (3) female dominance rank, (4) female fertility, and (5) the number of female coalitionary partners on the timing of female tolerance. In these models, the day of tolerance is the dependent variable, while the female characteristics are predictor variables, and the introduction (A, B, or C) is entered as categorical factor in the models. We employed the following stepwise forward selection procedure to arrive at the best fitting model. We

started by testing each of the five predictor variables (age, inverse age, female rank, female fertility and number of coalitionary partners) separately, to see which of these predictors explained the highest percentage of variance (R^2) in the dependent variable: 'day of tolerance'. This turned out to be the variable 'inverse age'. In the next step, we added each of the other four variables (age, rank, fertility, and coalitionary partners) separately, and used F tests to see whether each of these variables would add significantly in explaining the variance (R^2) in 'day of tolerance'. It was found that none of these four variables had an additional effect. Normality and homoscedasticity of the residuals of the best-fitting model were visually checked as well as tested via the Shapiro-Wilk test and the Levene's test, and Cook's distance was smaller than 0.5 for all data points.

One individual from introduction C, Mees, was an outlier and was left out of this analysis. Mees was maternally deprived during childhood, showed impaired social behaviour in the social group, and responded to the new male with extreme fear. As the other females in our study grew up in social groups with their mother present, we considered Mees not representative of normal social behaviour during male introductions. The results were in the same direction when taking Mees into account during our analysis, but less significant or non-significant. However, the main outcome and conclusions did not change.

Secondly, we analysed the changes in female behaviour during the increase of female tolerance of the male. To analyse these changes independent of the duration of the introductions, we calculated the rates of female behaviour towards the male for each period of days in which the same number of females had tolerated the male. We refer to these time frames with constant degree of tolerance as 'tolerance bins'. The percentage of females tolerating the male is a measure that is independent of the duration of the introduction. Comparing rates of female behaviour across tolerance bins shows how behaviour changes when additional females tolerate the male. There were 12, 8, and 9 tolerance bins for introductions A–C, respectively. The number of observation days within each bin varied from 1 to 20 days, with an average of 3.38 ± 4.21 days.

We divided the sum of occurrences of female aggression, unprovoked submission, affiliation, and mating access towards the new male, by the observation time within each tolerance bin (i.e. calculating behaviour rates per hour for each tolerance bin). Subsequently, these rates of female behaviour towards the male were logarithmically transformed using the natural log. General linear models, each with separate intercepts and separate slopes for the three introductions, were fitted to test whether female aggression, submission, and affiliation towards the male, as well as male mating access linearly increased or decreased with integration progress (i.e., the percentage of females tolerating the male). Female behaviour was the dependent variable, while tolerance percentage was entered in each model as predictor variable, and the introduction (A, B, or C) was entered as categorical factor. The weight of each data point was set to the number of days a tolerance bin lasted. Normality and homoscedasticity of the residuals of the models were visually checked as well as tested via the Shapiro-Wilk test and the Levene's test, and Cook's distance was smaller than 0.5 for all data points.

All tests were two-tailed with critical significance level set to 0.05. *R* version 3.2.3 was used for statistical analyses. For the general linear model we used the packages *lm* (to fit and test general linear models) and *anova* (to compare different models). Graphics were made with *R* version 3.2.3.

2.7. Ethical approval

All applicable national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

3. Results

3.1. Male behaviour

All three males were successfully introduced into their respective new group and obtained the alpha position. No clear differences in aggression (average ± SEM: 11.11 ± 0.38), submission (average ± SEM: 0.70 ± 0.70), and affiliation (average ± SEM: 1.96 ± 0.69) were found between the three new males during the first 5 h of their introductions (Fig. 1a–c). In contrast, a clear difference between the three males was visible in mating access (average ± SEM: 26.24 ± 10.11). During the first five hours, the mating rate of the male in introduction C was more than three times higher than the mating rates of the males in introductions A and B (Fig. 1d).
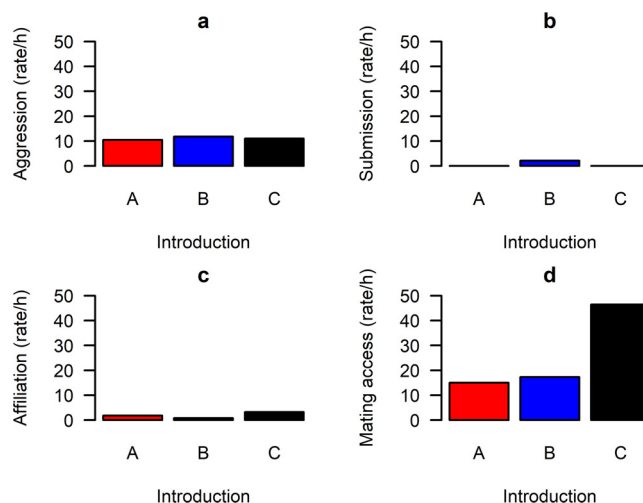

Fig. 1. The rates of new male to resident female aggression (a), submission (b), affiliation (c), and mating access (d) within the first 5 h of the introduction. There are no obvious differences between the males in aggression, submission and affiliation, while the male in introduction C mated more than twice as much as the other two males.

3.2. Female tolerance

A fast increase in the percentage of females tolerating the male during the first 7 days of contact was seen in all three introductions. Thereafter, it took longer before additional females tolerated the new male. At the end of the observation period, 100% (introduction A), 92% (introduction B), and 83% (introduction C) of the females tolerated the male (Fig. 2).

We investigated whether female tolerance of the new male depended on female age, dominance rank, fertility, and the number of female coalitionary partners. Female age (GLM, N = 40, R^2 = 0.262, p = 0.011), the inverse of female age (1/age) (GLM, N = 40, R^2 = 0.545, p < 0.001), and female dominance rank (GLM, N = 40,
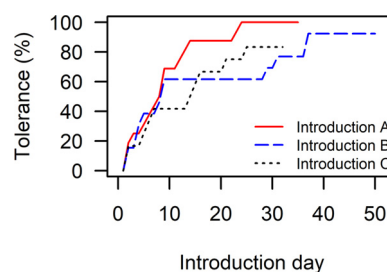

Fig. 2. The increase in female tolerance percentage during introduction A (solid red), introduction B (blue stripes) and introduction C (black dots).

Table 1
The effect of individual female characteristics on the timing of female tolerance during each of the three (or two) introductions, and the overall explained variances (R^2). The second model (Inverse age) is the best-fitting model.

Dependent variable: Day of tolerance Model	Introduction A N = 16	Introduction B N = 13	Introduction C N = 11	Variance explained N = 40
Age	t = -3.425 **	t = -2.556 *	t = -3.535 *	R^2 = 0.262 *
Inverse age	t = 3.423 **	t = 6.402 ***	t = 4.483 ***	**R^2 = 0.545 *****
Dominance rank	t = 0.990	t = 3.074 **	t = 2.139 *	R^2 = 0.238 *
Coalition size	t = -1.434	t = -1.070	t = -0.272	R^2 = 0.072
	N = 16		N = 11	N = 27
Fertility	t = -0.126	x	t = 1.025	R^2 = 0.055

* $0.05 \geq p > 0.01$, ** $0.01 \geq p > 0.001$, *** $p \leq 0.001$.
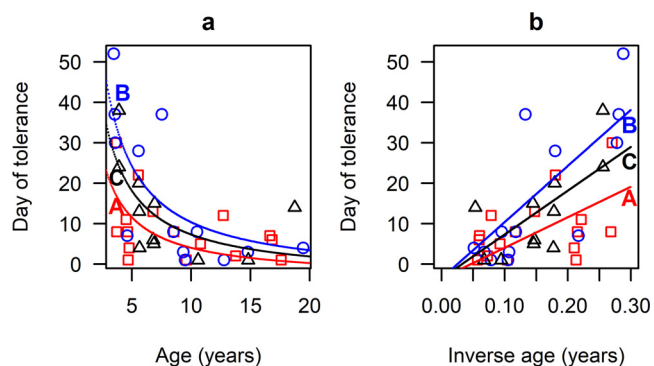

Fig. 3. The inverse of female age is the best predictor of the timing of female tolerance during introductions A (red squares), B (blue circles), and C (black triangles). Older females tolerated the new male sooner than younger females (a). The predicted lines are fitted by GLMs with inverse age as predictor and include different slopes, but not different intercepts (b).

R^2 = 0.192, p = 0.050) significantly correlated with the timing of female tolerance during the three introductions (Table 1). The number of female coalitionary partners (GLM, N = 40, R^2 = 0.072, p = 0.434)

and female fertility (GLM, N = 27, R^2 = 0.055, p = 0.506) did not significantly influence the timing of female tolerance (Table 1). The inverse of age is the best predictor of female tolerance, explaining almost 55% of the variation in the timing of female tolerance (Table 1 and Fig. 3). Adding age, dominance rank, the number of female coalitionary partners, or fertility to this best-fitting model did not add significantly to the model. Each of the extended models was compared with the best-fitted model yielding non-significant F-tests: F(3,33) = 1.07, 1.20, 1.64 and F(2,22) = 0.08, respectively (p ≥ 0.20; Table 2). We also tested whether the model with separate intercepts for each of the introductions added significantly to the best-fitting model (which has the same fitted value for the intercept irrespective of the introduction); this was not the case: F(2,34) = 0.96 (p = 0.39). So, the final, best fitting model has inverse age as the only predictor of the timing of female tolerance and has different slopes for the three introductions, but not different intercepts (Fig. 3b). In conclusion, older females tolerated a new male significantly sooner during introductions than did younger females. Other female characteristics did not add significantly to this inverse-age effect on the integration progress.

3.3. Changes in behaviour with integration progress

Female aggression, submission, affiliation, and male mating access are expected to change as integration of the new male progresses. For
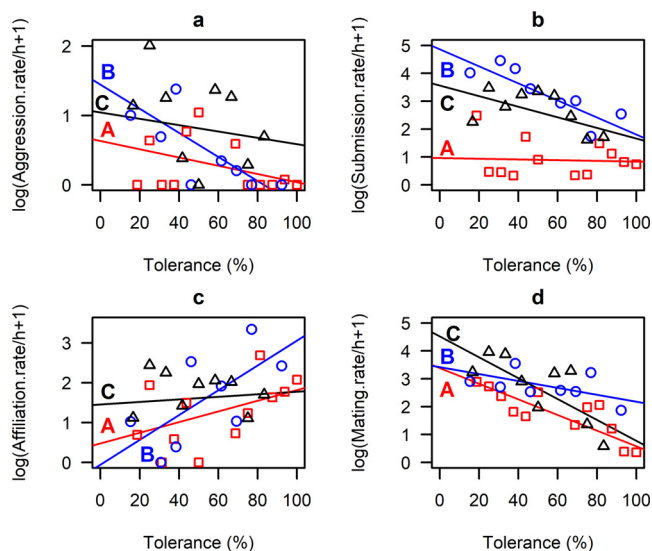
Table 2
The additional effect of each of the characteristics *Age, Dominance rank, Coalition size and Fertility* on the timing of female tolerance during the three (or two) introductions, when adding each of these characteristics to the best-fitting model (Inverse age as only predictor). The last column presents *F* values showing that none of these characteristics adds significantly to the best-fitting model.

Dependent variable: Day of tolerance Model	Introduction A N = 16	Introduction B N = 13	Introduction C N = 11	Variance explained N = 40	F test
Age added	t = 2.515 *	t = 4.386 ***	t = 3.137 **	R^2 = 0.586 ***	F(3,33) = 1.08
Inverse age	t = 1.531	t = 0.844	t = 1.166		
Age					
Dominance rank added	t = 2.437 *	t = 4.553 ***	t = 2.404 *	R^2 = 0.576 ***	F(3,33) = 0.80
Inverse age	t = 0.573	t = 0.636	t = 1.398		
Dominance rank					
Coalition size added	t = 2.683 *	t = 6.510 ***	t = 3.413 **	R^2 = 0.604 ***	F(3,33) = 1.64
Inverse age	t = -0.784	t = -2.067 *	t = 0.274		
Coalition size					
	N = 16		N = 11	N = 27	F test
Fertility added	t = 2.516 *	x	t = 3.294 **	R^2 = 0.393 *	F(2,22) = 0.08
Inverse age	t = 0.340	x	t = -0.172		
Fertility					

* $0.05 \geq p > 0.01$, ** $0.01 \geq p > 0.001$, *** $p \leq 0.001$.

Fig. 4. The changes in female aggression (a), submission (b), affiliation (c), and mating access (d) to the new male during introduction A (red squares), B (blue circles), and C (black triangles). The predicted lines are fitted by GLMs that include different slopes and different intercepts.

each of these behaviours we fitted a general linear model (GLM) with female tolerance percentage (i.e. the tolerance bins) as a predictor, while including separate intercepts and slopes for the three introductions. Female aggression (GLM, N = 29, R^2 = 0.486, p < 0.001; Fig. 4a) and submission (GLM, N = 29, R^2 = 0.836, p < 0.001; Fig. 4b) towards the new male decreased significantly with integration progress. Female affiliation towards the male increased significantly as integration progressed (GLM, N = 29, R^2 = 0.413, p < 0.001; Fig. 4c). Male mating access decreased significantly with integration progress (GLM, N = 29, R^2 = 0.739, p < 0.001; Fig. 4d). For each of the four behaviours, all changes were in the same direction for all three introductions. However, not all slopes differed significantly from zero and effect sizes differed between the introductions (Table 3). In conclusion, a general decrease in female aggression, submission, and male mating access was observed with integration progress, while female affiliation increased.

4. Discussion

This study identified male and resident female behaviour during male introduction in captive rhesus macaques and showed that female age is the paramount female characteristic that influences a female's attitude towards new males.

4.1. Male behaviour

The initial, relatively high levels of aggression and low levels of affiliation by the three new males is in accordance with previous research (captivity: Bernstein et al., 1977; Rose et al., 1972; wild: Alberts et al., 1992; Bercovitch, 1997; Marty et al., 2017; Teichroeb et al., 2011; Zhao, 1994). Behavioural differences between the new males may explain variances in female behaviour (Marty et al., 2016; Van Noordwijk and Van Schaik, 2000, 1985). In our study, however, no clear differences were observed between the three new males in initial aggressive, submissive, and affiliative behaviour towards the resident females. Nonetheless, the male in introduction C had much higher mating access than the other two males. However, we consider male mating access a result of both new male and resident female behaviour, since female cooperation is required. Therefore, our results indicate that there is only minor variation in the males' behaviour. Conversely, we have seen variation in female aggression, submission, and affiliation levels towards the different males. Thus, our data do not show an obvious relation between the male's behaviour at the start of the introduction, and the subsequent integration process. Unfortunately, the current number of introductions was too low to produce any meaningful statistics on the possible effect of male behaviour on female behaviour during introductions. Either a male's initial behaviour may not affect how females respond, or the link between male and female behaviour may be less straightforward.

4.2. Introduction and integration Success

Linking the integration success (the final female tolerance percentage) to the introduction success (the caretaker's decision to leave the male in the group permanently) can have important implications for introduction management. All three introductions were successful while the final percentages of female tolerance of the male were 83, 92 and 100%. This shows that a successful introduction is not tantamount

Table 3
The variance (R^2) in female social behavior towards the new male explained by integration progress (i.e. % of female tolerance) in the four GLMs. Per introduction the fitted values of the separate intercepts and slopes are given, as well as their statistics.

Predictor variable: % female tolerance Dependent variable		Introduction A N = 12	Introduction B N = 8	Introduction C N = 9	Variance explained N = 29
Aggression	Intercepts	0.634, t = 1.702	1.445, t = 3.716 **	1.044, t = 3.130 **	R^2 = 0.486 ***
	Slopes	−0.006, t = -1.233	−0.017, t = -2.708 *	−0.005, t = -0.809	
Submission	Intercepts	0.946, t = 1.930	4.859, t = 9.314 ***	3.563, t = 7.964 ***	R^2 = 0.836 ***
	Slopes	−0.001, t = -0.192	−0.031, t = -3.661 **	−0.019, t = -2.510 *	
Affiliation	Intercepts	0.476, t = 0.835	−0.055, t = -0.083	1.453, t = 2.848 **	R^2 = 0.413 ***
	Slopes	0.013, t = 1.815	0.031, t = 3.269 **	0.003, t = 0.371	
Mating access	Intercepts	3.386, t = 5.959 ***	3.421, t = 5.766 ***	4.520, t = 8.886 ***	R^2 = 0.739 ***
	Slopes	−0.028, t = -3.810 ***	−0.012, t = -1.308	−0.038, t = -4.357 ***	

* 0.05 ≥ p > 0.01, ** 0.01 ≥ p > 0.001, *** p ≤ 0.001.

to a 100% successful integration. Thus, tolerance by a subset of females seems sufficient to decrease the risk of attack by female coalitions drastically (as assessed by the experienced caretakers). Unfortunately, behavioural data on unsuccessful introductions are lacking, making it difficult to identify a possible female tolerance threshold (i.e. minimum percentage of tolerating females) for successful introductions. We aim to fill this knowledge gap in future studies, since approximately 1 in 5 introductions are unsuccessful (unpublished data from the BPRC, collected between 2004 and 2011, N = 43).

4.3. Female characteristics

Female age, dominance rank, fertility, and the number of female coalitionary partners were expected to affect the timing of female tolerance of the new male. However, only female age determined tolerance: younger females tolerated the male later than older females. The inverse of female age was a better predictor than age itself. Thus, the relationship between female age and tolerance is not linear but negative exponential (Fig. 3a). Young individuals were slow in tolerating the new male, but females above a certain age tolerated the male relatively fast. When looking at our data, the breakpoint for quick tolerance may be around the age of 7 years. These females had less experience with male introductions. All of the 7 years and older females had experienced a male introduction before (75% during adulthood, 25% as juvenile), while none of the younger females did. This shows that experience with male introductions may be an important factor influencing a female's attitude to an unfamiliar male and could explain the observed effect of the inverse of age. Moreover, younger females may be more fearful during male introductions, since younger individuals generally behave more submissively than older ones (Bernstein and Ehardt, 1985). Lastly, the effect of age on female tolerance of the male may overrule the effects of female dominance rank and fertility found in other studies (Kawai, 1960; Zaunmair et al., 2015). Dominance rank and fertility possibly come into play after female tolerance, determining male social and mating access at a later stage.

4.4. Changes in female's behaviour towards the new male

Changes in a female's behaviour towards the male during the introduction represent the female's role in the integration process.

4.4.1. Aggression

Female aggression to the new male indicates the resistance a male encounters during an introduction. As expected, female aggression towards the new male decreased with integration progress, possibly due to increased familiarity. A decrease in aggression with integration progress is in accordance with previous studies on rhesus macaque introductions (Bernstein et al., 1977; Bernstein and Mason, 1963). In contrast to our findings, female aggression towards the male was absent when creating a one-male, multi-female lion-tailed macaque (*Macaca silenus*) group (Zaunmair et al., 2015). Lion-tailed macaques are a relatively tolerant species, while rhesus macaques are considered rather intolerant (Thierry, 2000). This indicates there are species-specific responses during introductions (Clarke et al., 1995).

We found that aggression during introductions can also differ between introductions within the same species. In two of the three introductions (A and B), the rate of aggression was virtually zero after 75% of the females tolerated the male. In the third introduction (C), however, aggression did not peter out, while at the same time the percentage of females that tolerated the male did not exceed 83%. Although the male in group C was successfully introduced, there may still have been resistance present among the females against this male, possibly with long-term implications. Indeed, after several months this male had to be removed from the group due to high aggression levels, while the males in the other two introductions stayed in the group for at least three years (BPRC animal register). Thus, very low female

aggression levels at the end of the introduction may signal long-term group stability. However, no hard conclusions on possible long-term effects of remaining aggression can be drawn from comparing these three cases only.

4.4.2. Submission

A decrease in female submission during an introduction may indicate acceptance of the new male's dominant position. As expected, female submission decreased significantly with integration progress. This is in accordance with results from a previous, descriptive, study (Bernstein and Mason, 1963). However, when comparing the three introductions, only significant decreases in submission were visible during introductions B and C. During introduction A, female submission remained low and was the lowest of all three introductions (Fig. 4b). This variation may be due to differences in female characteristics (e.g. personality) between the three groups or due to male's behaviour.

Submission levels were low at the end of the three introductions. These low rates of female submission towards the male may signal that the females no longer perceive him as a threat. This is an important step in the tolerance of the new male and thus a crucial aspect of a successful integration. Indeed, introduction A (with the lowest female submission rate) had the fastest female tolerance increase, and the management decision to leave the male in the group full-time was made after only 12 days of contact. Although low submission rates may indicate short-term safety, they appear unrelated to long-term aggression and affiliation patterns (e.g. introduction C). Still, these results suggest that animal caretakers can use a low rate of female submission towards the introduced male as a signal that the male can stay in the group full-time.

4.4.3. Affiliation

Female affiliation towards the new male increased significantly during the course of the three introductions. It has been suggested that affiliation replaces aggression during introductions (Bernstein et al., 1977). Consistent with this idea, the introduction with the largest decrease in aggression, introduction B, also showed the largest increase in affiliation, and the introduction with the smallest decrease in aggression, introduction C, also showed the smallest increase in affiliation, suggesting that these two social strategies may depend on each other.

An increase in affiliation may indicate that the females started bonding with the male during the introductions. Initial social interactions are important in the formation of social bonds on the longer term (Dunayer and Berman, 2017; Evers et al., 2015). The observed affiliation may thus eventually provide the male with a position in the female social network, in which rhesus macaque males usually become well integrated (Hill, 1990). Possibly, less or weaker social bonds were formed in introduction C. The long-term effects of the minimal affiliation in this group remains unknown, but the existence of weak social bonds may have led to the removal of the male from the group a few months after the introduction (see above). However, we cannot statistically support this supposition due to our low sample size.

4.4.4. Male mating access

Male mating access decreased with integration progress, after initial high copulation rates. Female mating interest may decrease with pregnancy; however, female rhesus macaques also mate when pregnant (Loy, 1971). Alternatively, the Coolidge effect, i.e. when an individual loses sexual interest in a mating partner that has been available for a longer period of time (Dewsbury, 1981), may explain the decrease in mating access. Indeed, primates prefer novel mating partners (Inoue and Takenaka, 2007; Manson, 1995). The possible existence of a Coolidge effect in rhesus macaques can have important management implications for captive breeding colonies. Mating interest, and therefore reproductive success, may decrease when the male has spent more time in the group. When reproduction rates are lower than desired, likely after a few years, the introduction of a new breeding male may restore mating interest and increase reproduction.

5. Conclusion

To summarize, female aggression and submission towards the new males decreased as integration progressed. Female affiliation increased, and mating rates decreased. In general, the integration progress and the changes in social behaviour were consistent across the introductions, indicating a general pattern in female behaviour during male introductions. Low levels of female submission can play an important role in determining when it is safe to leave a male in the group full-time. Low female-male aggression levels, on the other hand, may indicate long-term group stability. Although a general pattern in female behaviour is discernable, differences in effect sizes and significance within this general pattern cannot be ignored. This may be due to female characteristics, but not to differences in male behaviour. Overall, we conclude that female behaviour can provide valuable information about the integration process during male introductions, that can be helpful in management decisions.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.applanim.2018.07.006.

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