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Influence of female coalitionary aggressive behavior on the success of male introductions to female groups of rhesus macaques (*Macaca Mulatta*)

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Abstract

Migration patterns of wild rhesus macaque males are often mimicked in captivity by introducing unfamiliar males to female groups every few years. This strategy prevents inbreeding and has been shown to encourage group stability once males are fully integrated. The current study focused on female coalitionary aggressive behavior directed toward males during introductions to describe factors that predict its frequency and any relationship with introduction success. Observational data (755 h) were collected during eight introductions of male cohorts (3–7 individuals) to established female groups (14–39 breeding-age females). Female coalitionary aggression (FCA), defined as four or more females simultaneously attacking an individual male, was recorded 114 times and occurred during all introductions. Data showed that male groups with alpha males who aggressed females during the coalitionary events were more likely to be successfully integrated than those with alpha males who did not retaliate against females. Stepwise multiple regression analyses of individual females (N = 183) revealed that females from larger groups and older females were more likely to be involved in coalitionary aggression, while rank, family size and number of matrilines in the group did not play a role. A rating system of the severity of FCA events revealed male groups receiving more severe FCA were less likely to be successfully introduced, and larger male groups received more severe FCA than did smaller groups of males. Based on these data, it is recommended that colony managers expect FCA to occur during introductions, especially with older females and larger groups. Colony managers should monitor the alpha male’s response to FCA, as well as the severity of the FCA since those factors may predict introduction success.

Keywords

Coalitions; Introductions; Aggression; Rhesus macaque; Breeding groups

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Declaration of Competing Interest

The authors report no declarations of interest.
1. Introduction

Rhesus macaques (*Macaca mulatta*) live in large multi-male, multi-female groups with female philopatry (Gouzoules and Gouzoules, 1987) and male dispersal (Colvin, 1986; Lindburg, 1969). Wild rhesus macaque groups typically are comprised of 40–300 (Rawlins and Kessler, 1986) members and are composed of several multi-generational matrilines (Wrangham, 1980; Gouzoules and Gouzoules, 1987). Most, but not all, male rhesus periodically migrate to new social groups throughout their adult lives (Neville, 1968; Lindburg, 1969; Koford, 1963, 1966; Melnick and Pearl, 1987; Chapais, 1983). Male rhesus migration typically begins at puberty and then about every 2–5 years or more often as males age (Melnick et al., 1984; Lindburg, 1969). These males migrate as individuals or in small bachelor groups (Melnick et al., 1984; Gacho-Neveu and Menard, 2004). Since female rhesus remain in their natal groups for life in this matrilineally-organized society, implementation of a similar male migration behavior pattern in captivity prevents inbreeding, but also creates a social system in which females and resident males are repeatedly exposed to novel males attempting to enter their groups.

Many captive primate facilities aim to mimic the natural lifestyle of these highly social primates by housing them in large social groups. Colony managers may then simulate natural migration patterns by introducing unfamiliar males to groups of females and their offspring. These introductions are often performed in the breeding season, when females’ receptivity is expected to be the highest (Vandenbergh and Vessey, 1968; Rawlins and Kessler, 1985). Although male introductions or some other method of minimizing inbreeding is a necessary component of captive breeding group management, introductions of unfamiliar animals can be risky due to the xenophobic nature of rhesus macaques that can result in wounding and trauma exposure (Singh and Gupta, 1980; Bernstein et al., 1974). Thus, understanding factors that contribute to the safe and successful integration of new males to stable social groups is critical for the management of captive breeding groups of rhesus macaques.

Sexual dimorphic traits in rhesus macaques may be an important consideration, as a weight differential of about five kilograms typically exists between captive males and females (Leigh, 1994), and adult males have much larger canine teeth than females (Lauer, 1975; Bernstein and Ehardt, 1985; Beisner et al., 2012). However, social factors may play a more important role in new male introduction success, as the matrilineal nature of rhesus social organization creates lifelong social bonds among females, and aggressive cooperation among the smaller-sized females often allows them to overcome the inherent advantages of their larger-sized male opponents (Archer, 1988). Female rhesus monkeys are highly protective and defensive of both their kin (Isbell and Young, 2002) and non-kin group members (Chapais, 1992), and they often exhibit aggressive behavior towards unfamiliar conspecifics (Bissonnette et al., 2015), creating a risk-prone and stressful environment (Bernstein et al., 1977; Rose et al., 1972) for both incoming males and for the resident females during introductions.

One component of female rhesus macaque social behavior that may be important for the success of new male group introductions is their formation of long-term coalitionary
alliances with relatives and mutualistic coalitions with non-relatives (Sterck et al., 1997). Although these patterns evolved in the wild due to food competition and as a method of decreasing predation risk, coalitionary aggression in females is also common in captivity and serves as reinforcement of the dominance hierarchy (Chapais, 1992). Female coalitionary aggression (FCA) is a form of cooperation in a competitive or aggressive context in which two or more individuals join to attack a common conspecific target (Harcourt and de Waal, 1992). In many primate species in both captivity and the wild, females have worked together with this aggressive strategy to delay or prevent some males from entering their social groups (olive baboons and Japanese macaques, Packer and Pusey, 1979; long-tailed macaques, Van Noordwijk and Van Schaik, 1985; rhesus macaques, Bernstein et al., 1977; Neville, 1968). While FCA of related and unrelated females against unrelated, dominant and non-peripheral males has been reported in the Cayo Santiago population (Chapais, 1986), it remains unclear whether FCA is an important contributor to the outcome of new male introductions in captivity. Although Rox et al. (2018; 2021) did postulate that female rhesus behavior is probably instrumental in the success of introductions, FCA has not been examined within the framework of introductions. Thus, the objective of the current study is to describe FCA events during the introduction of new groups of males to established groups of captive female rhesus macaques, and to ascertain whether FCA was related to the success or failure of the introductions. We hypothesized that higher rates of FCA and more severe FCA would be associated with unsuccessful introductions. We also conducted exploratory analyses to determine what factors predicted a) male response to FCA and b) female involvement in FCA.

2. Methods

2.1. Subjects and Housing

The current study was conducted at the Yerkes National Primate Research Center (YNPRC) Field Station in Lawrenceville, GA, USA from September 2017 through December 2019. The subjects were 149 adult female rhesus macaques, living in six different social groups, and 26 adult male rhesus macaques, living in five groups, who were introduced to them. Three of the male groups and two of the female groups were studied in two different introduction years (see Table 1). For this reason, the total subject pool of adult females for statistical analysis was 183 (34 were assessed twice) and for adult males it was 38 (12 were assessed twice). All adults were uniquely tattooed, and some were uniquely dye-marked to facilitate individual identification. The female social groups consisted of 14–39 adult females (ranging in age from three to 22 years) and their non-adult offspring (20–55 infants and juveniles per group), with total group sizes ranging from 34 to 94 individuals. Most female offspring remain in their natal group throughout their life, and male offspring are removed around three years of age. Two of the 183 females were later learned to have been pregnant at the start of the introductions in their groups. The adult male groups consisted of three to seven adult males ranging in age from five to 14 years. Their weights ranged from 7.8–15.9 kg at the onset of introduction. These male groups had lived together for 1.5 to five years and were considered socially stable by Colony Management staff based on routine behavioral observations.
The rhesus macaque breeding colony at the YNPRC is primarily maintained in large breeding groups (18–170 animals), comprising multiple multi-generational matrilines, and housed in 0.06- to 0.38-acre outdoor compounds with attached indoor enclosures. Social densities ranged from 0.07 to 0.11 individual monkeys per square meter of space. The indoor enclosures are divided into two units: a smaller capture unit connected to a larger living space by removable doors. During introductory events, the new male groups were housed in one of two locations: an introduction enclosure connected to the outdoor compound by shared chain-link fencing (see Bailey et al., 2021) or in run housing located elsewhere at the facility.

The YNPRC facility and its programs are fully AAALAC-accredited. Procedures involving all animals were approved by the Emory University IACUC and were conducted in accordance with USDA Animal Welfare Regulations (9 CFR ss 3.129), The Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and institutional policies. All animals had continuous access to fresh drinking water and unrestricted access to food. Routine enrichment provided to all animals included fresh produce, climbing and playing structures, foraging devices, and manipulanda.

2.2. Introduction Procedure

Eight male group introductions into female social groups that were part of YNPRC’s regular breeding management program were studied. The male groups experienced one of two housing situations (for details see Bailey et al., 2021). Four groups experienced the traditional Yerkes introduction which involved moving the male group from run housing into the larger part of the indoor enclosure which was attached to a compound containing females during the day and returning them to their run housing each afternoon. Movements within the facility occurred via individual transfer boxes; adult males were previously trained to enter and exit a box when prompted. While housed in the indoor enclosure, the males could see the females who entered an adjacent, smaller indoor area, as well as have limited physical contact with those females through chain-link fencing. The other four groups experienced a different process using an introduction enclosure that was permanently attached to the outdoor compound where females were housed. This introduction enclosure can house multi-male groups 24 -hs a day, for long periods of time and allows constant protected contact interactions between the new males and the females through chain-link fencing if they choose.

All eight introductions were performed the same way other than this housing difference. The introductions were carried out by experienced Colony Management staff members between September and January, the rhesus breeding season at the YNPRC. Adult males already residing in the female social group were permanently removed prior to the start of the introductions; the length of time they lived without males varied from one to 48 days depending on the stability of the female social group without males present and other management factors. In both introduction situations, the animals remained in the protected contact stage for one to eight days. Colony Management staff decided when to proceed to the full contact stage of the introduction based on the animals’ behavior and receptiveness to each other, staff availability, weather, and other management factors. To make decisions
about carrying forward with steps in the introduction process, Colony Management staff looked for low levels of aggression among the males and between the males and females, appropriate unidirectional status signaling, lack of fearfulness from low-ranking males, and affiliative or sexual interest, especially from high ranking individuals. Due to large variations in Yerkes’ breeding group sizes, matriline sizes, male group age and experience, the number of introductions occurring each season, etc., there are no strict rules that apply to all introduction scenarios. Each is evaluated independently based on the specific animals involved. Prior to the full contact introduction, the male groups were given at least three opportunities to explore the outdoor compound without the females present (30–60 min each time) to give them a chance to learn where food and water were located, boundaries of the space, etc. Once deemed appropriate, the males were introduced to the female group with full contact and were only moved back to the protected contact areas outside of the hours of introductory events. On the first day of the introduction, the females were housed initially in their indoor enclosure, the males were released to the outdoor enclosure, and the females were then released in two to five sets of adults and their infants, depending on the size of the group. This was to prevent the males from becoming initially overwhelmed by the entire female group at once. As the introduction progressed, an experienced staff member decided when the animals were given additional access to the full indoor and outdoor enclosure. The amount of time the males and females had full contact gradually increased each day of the introduction, based on the decision of the Colony Management staff. This time ranged from 0.5 to 2 h to start and 4.5–7.5 h by the end of the introduction. At this point the male group began spending the night and living full time with the female group or the introduction was ended by Colony Management and Veterinary staff due to social incompatibility or recurring traumas. Once in the full contact stage, animals had access to the full indoor and outdoor enclosures except during cleaning of the indoor area or during inclement weather. The duration of the introductions ranged from 10 to 128 days.

Introductions deemed successful were those in which an adult male-to-adult female ratio of at least 1:10 was maintained for at least 28 consecutive days and nights with males and females living in the same space. This is similar to a 4-week timeframe Rox and colleagues (2018) used to define successful introductions.

2.3. Behavioral Observations

Four observers recorded behavioral observations during the introductions with two or three observers present at one time. Inter-rater reliability was tested before any data were collected. All observers achieved a reliability score of 100% agreement in animal identification and 95% agreement on all behaviors. Data collection reliabilities were calculated using Krippendorff’s alpha and ranged between 0.85–1.00 across all observers (Krippendorff, 2011) for individual behaviors. Reliability tests were conducted during the animals’ most active periods, typically around the provision of enrichment. Three groups were observed September through December 2017, three October through early January 2018, and two October through December 2019. Data were collected Monday through Friday between the hours of 0700–1600 for approximately 24 h per week with a total of 755 h across all eight introductions. Observations were not performed on days of extreme weather or temperatures due to the inactivity of the animals.
Observation hours for each introduction varied depending on behavior of the animals, inclement weather, other ongoing facility introductions, and staff availability. Each individual male may have a different number of observation hours due to injuries or illnesses requiring treatment and/or hospitalization away from the group. In order to account for this, an FCA hourly rate was developed in which the total number of coalitionary aggressive events an individual participated in was divided by the total number of observation hours for which that individual was present.

An event sampling method was used to collect data on agonistic interactions which were recorded as a chronological record of the initiator and recipient of each aggressive interaction within a conflict event. Aggressive behaviors were categorized in increasing levels of severity (mild, moderate, severe) (see Table 2 for operational definitions). Type of aggressive interaction (i.e. direct, intervention, redirect), number of participants on each side of the aggression, total conflict duration, and any other contextual behaviors (i.e. interventions, status-signaling, affiliative behaviors occurring during the conflict), were also recorded. An FCA event was recorded when a coalition of four or more adult females aggressed against one adult male recipient. The actual number of female participants in the larger coalitionary events recorded may be somewhat under-reported, as observers may have had difficulty recording all participants. Agonistic events containing coalitions were filtered from all other events and analyzed for this study.

### 2.4. Attribute Data Collection

Data were collected on group and individual attributes. The rank of each adult female and male was determined based on Colony Management staff observations of silent bared teeth expressions, displacements, and withdrawals, and followed the typical linear hierarchical structure found in rhesus macaque groups. All other attributes (female and male group size, the number and size of matrilines, the age of each adult female, the number of offspring living in the group for each adult female) were gathered via the YNPRC electronic animal records system.

### 2.5. Data Analysis

We compared the mean FCA rates across the two introduction methods (traditional versus introduction enclosure methods) by an independent samples \( t \)-test.

Male group and individual factors (male group size, individual rank) were analyzed using point biserial and Pearson’s correlations. Correlations with \( p \) values of 0.05–0.10 are reported as trends, and those with \( p \) values less than 0.05 are reported as significant findings. We calculated a mean FCA rate for each male group and performed a point biserial correlation of mean FCA rate and introduction success, and Pearson’s correlations of individual male rank and male group size with FCA rate.

Female group and individual variables were analyzed to determine the relative contribution of the measured factors to the females’ involvement in FCA events. A stepwise linear regression including group size, the number of matrilines in the group, the size of each female’s matriline, female age, the number of offspring living in the group for each adult female, the number of days the female group was housed without males before the
introduction, and the familial rank of each adult female was performed to determine which
female-related factors are most important for predicting involvement in FCA events.

To more closely examine factors contributing to individual FCA events, a scoring system
was developed to assess severity including the conflict duration, the highest level of
aggression recorded during the event, the percentage of the adult females in the group
participating, and the percentage of matrilines represented in each FCA (see Table 3). The
four scores, each ranging from one to three, were summed for a total severity index which
could range from three to 12. A point biserial correlation was performed on each male
group’s total combined severity index and introduction success, and a Pearson’s correlation
was performed on male group size and total combined severity index.

Aggression typically decreases over the course of a male introduction (Bernstein et al.,
1977). Therefore a Pearson’s correlation was conducted on the number of FCA events per
day for each male group and the day of introduction. A further correlation of the total
combined severity scores each day and the day of the introduction was run to measure
whether severity of the events changed over time. Pearson’s correlations were performed
to determine if the severity of events over time differed for successful versus unsuccessful
introductions.

Finally, an analysis of male behavioral response was performed. Each alpha male’s
responses to FCA events were summed to determine the percentage of times he submitted,
returned aggression, or gave no response to FCA events directed to him. A point biserial
correlation was performed on each alpha male’s percent of responses involving returned
aggression to the females and introduction success.

3. Results

3.1. Success of Male Introductions

Of the eight introductions assessed, four were deemed successful and four unsuccessful
by our definition of achieving an adult male-to-female ratio of at least 1:10 that was
maintained for at least four weeks. Once established, the successful groups at our center
generally remain together for three to four years until the next male introduction occurs.
The introduction method did not significantly affect the mean male group FCA rate which
allowed us to collapse all of the data across introduction method (t(6) = 1.891, p = 0.107).
As noted in Table 1, Female Group 1 had a successful introduction in 2017 and the males
lived with the females for about one year, but in 2018 these males were removed and a
different cohort of males was introduced to maximize breeding success in our colony. After
Female Group 3 experienced an unsuccessful multi-male introduction in 2017, a single
male was introduced instead. The following year he was replaced with a multi-male group,
which is more desirable for the breeding program. Although Male Group D was successfully
introduced in 2018, they were removed and introduced to a different group in 2019 so that
female group could experience a multi-male group structure.
3.2. Rates and Characteristics of FCA Events

FCA events occurred in all eight male introductions a total of 114 times. Seventy-three percent of females from all groups participated in at least one FCA. The number of participants in a single event ranged from 10% to 57% of the adult females, with a mean of 20.2% of them aggressing a male. The percentage of matrilines participating in a single event ranged from 9% to 100%, with a mean of 47.5%. Most coalitionary events involved aggression of moderate intensity (52.6%), with 33.3% of mild intensity and 14.0% severe intensity. The mean duration of all recorded FCA events was 60.2 s. The rate of FCA received by individual males ranged from 0 to 0.23 per hour. FCA rates for female involvement ranged from 0 to 0.22 per hour. Only four males in our study sample of 26 were not targeted by FCA at least once, and all four were the lowest ranking male within their cohort.

3.3. Relationship of FCA and Its Severity with Introduction Success

The rate of FCA directed to alpha males and introduction success trended towards a significant negative point biserial correlation ($R^2 = -0.44; p = 0.07$), with alpha males in successful introductions having received fewer FCA events. Mean male group FCA rates were not significantly correlated with introduction success ($p > 0.10$). Individual male rank within his group and FCA rate also trended towards significance ($R^2 = 0.09; p = 0.07$), with higher ranking males tending to be targeted by females more often. Male group size and FCA rate were not significantly correlated ($p > 0.10$). There was only one introduction in which FCA events were the main cause of failure (male-male aggression contributed to the other failed introductions). In this case, the entire male group was removed to prevent instability within the male group and to preserve the entire male group for future introductions.

For all 114 events, the average FCA severity index was 6.30 out of 12. Male groups’ total sum of FCA severity indices and introduction success were significantly correlated ($R^2 = 0.54; p = 0.03$) with male groups that received more severely-rated FCA being less likely to be successfully introduced (Fig. 3). Male group size and FCA severity ratings were also significantly correlated ($R^2 = 0.73; p = 0.007$); larger male groups received more severe coalitionary aggression (Fig. 4). The number of coalitionary events per day and the day of the introduction were significantly negatively correlated ($R^2 = -0.44; p < 0.01$) with fewer FCA events occurring as the introductions progressed. The total combined FCA severity scores each day and the day of the introduction were not significantly correlated ($p > 0.05$). Neither the successful nor the unsuccessful groups showed a significant change in the FCA severity index over the course of their introductions as shown by Pearson correlations ($p$’s $> 0.05$).

Finally, alpha male responses to coalitionary aggression and introduction success were significantly correlated ($R^2 = 0.61; p = 0.02$) such that groups with alpha males who responded to FCA with aggression more often, were significantly more likely to be successfully introduced (Fig. 5).
3.4. Predictors of female participation in FCA

Stepwise multiple regression analyses of individual females (N = 183) revealed that females from larger groups ($\beta = .19$, $t = 2.70$, $p = .008$) (Fig. 1) and older females ($\beta = .37$, $t = 5.37$, $p < .001$) (Fig. 2) were more likely to be involved in coalitions, while rank, number of offspring in the group, matriline size, number of days the female group was housed without males, and number of matrilines in a group did not play a role ($p's > 0.10$).

4. Discussion

4.1. General Findings

The current study described and characterized the common behavior pattern of FCA events during male introductions to large groups of female rhesus macaques. Although most females from all ranks participated in at least one FCA, we found that older females living in larger groups were more likely to participate, and that male response to FCA is an important indicator of eventual introduction success. The fact that FCA occurred repeatedly (mean = 14.25 events per group) and during all of the introductions of males to resident female groups, highlights the xenophobic nature of female rhesus macaques and the importance of examining this under-studied behavior. However, to put this type of aggression in context, we recorded 3380 total agonistic events during the eight introductions we evaluated (during 2.874 h of observation) with a mean of 422.5 events per group (unpublished data), and only 114 were FCA events. The majority of FCA included moderate intensity aggression, and 14 % involved severe aggression, so this is a notable source of aggression that should be addressed. This aggression led to wounding of the males in at least three instances, with injuries ranging from multiple minor bite wounds to more severe crush trauma requiring veterinary intervention. Additional wounding events were possible, but observers could not always clearly see who inflicted wounds. We found that as the introductions advanced across days, the number of coalitionary events were reduced. This substantiates other literature showing that levels of aggression decrease over the course of an introduction (Lindburg, 1969) while levels of affiliation increase (Bernstein et al., 1977).

The lack of published descriptions of FCA during male introductions in captivity is surprising considering their frequency, and the high percentage of adult females and matrilines participating. We also know of no reports of similar FCA events occurring in the wild when new males are immigrating into groups of rhesus macaques. Instead these immigrations of one or two males at a time are described with relatively low rates of aggression and new males remaining on the periphery for several weeks before fully entering the groups (Lindburg, 1969). Although wounding rates for both sexes increase during the transfer period, actual fighting is not often observed (Lindburg, 1969). This contrast may be due to differences between the captive and wild situations. In wild rhesus groups, a large number of adult males remain in the resident group while new males attempt to join (Lindburg, 1969; Bercovitch, 1997). The immigrating males are integrated into the bottom of the hierarchy (Drickamer and Vessey, 1973; Berard, 1990; Bercovitch, 1997) and the literature does not note any strong aggressive responses of the resident males or females to these newcomers. If new males do face aggression, they have the option to flee the group and return another time or join another nearby group (Lindburg, 1969, 1971). In captivity,
our groups of three to seven males were introduced together to groups of females with no resident males in place. Perhaps this circumstance elicits FCAs in captive groups. Changes in captive introduction procedures that would better mimic the wild behavioral pattern, with one or two young adult males being slowly introduced to an intact group with some older, resident males would be interesting to cautiously test. The use of an introduction enclosure attached to the space where the intact group resides (Bailey et al., 2021) would allow such a procedure to be safely attempted.

4.2. Male Factors Contributing to FCA and Introduction Success

There was no relationship between the number of males being introduced and the FCA rate they received, so larger groups did not receive more frequent FCA, but below, the severity of that FCA is discussed. Two factors related to males and the frequency of FCA events did not quite meet our threshold for significance, but were close (p = 0.07), and due to the exploratory nature of this study, we think are worth discussing and deserve future investigation. The first of these is that higher-ranked males tended to experience more FCA events directed toward them. The only males who were not recipients of FCA events were the lowest ranking in their groups. The tendency of females to attack higher-ranking males more often may stem from behavior patterns of wild rhesus groups. Although not unheard of, it is unusual for a new male entering a group to immediately become the most dominant animal. Instead, immigrating males typically integrate from the bottom of the group hierarchy, and male rank is closely tied to tenure and age (Drickamer and Vessey, 1973; Berard, 1990; Bercovitch, 1997). Since the low-ranking males naturally rank below most females upon entry, there is no direct challenge to a female’s position in the dominance hierarchy as there is with the alpha male.

The second trend that is worthy of discussion is that alpha males who received less FCA were more likely to be involved in introductions deemed successful. This finding partially substantiates our hypothesis that higher rates of FCA would be associated with unsuccessful introductions, and the importance of how the alpha male is treated by females, is emphasized. Our correlational finding is not surprising because the frequency of FCA and whether the introduction continues to proceed are not independent events. In other words, more frequent FCA events would have been factored into decisions about whether males should be separated from the females permanently, hence contributing to an introduction failure. Nonetheless, monitoring the frequency of FCA events may be one metric colony managers could use as they gauge the progress of introductions.

Perhaps the most intriguing finding of the study is the interaction between alpha males’ responses to being aggressed by groups of females and introduction success. Groups with alpha males who responded to FCA by returning aggression were significantly more likely to be successfully introduced than those with alphas who submitted to the females or who lacked any obvious response. This finding is consistent with the observation that when adult males flee aggressive attacks, other group members often join in the chase (Bernstein and Ehardt, 1985). Rox et al. (2021) also believe that male response to female coalitions is key in their single-male introductions. They report that if a male “loses” fights against female coalitions, he will experience social defeat, and the introduction cannot

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continue. Since it is theorized that female coalitions serve the purpose of rank acquisition and rank maintenance (Chapais, 1992), a male who responds with aggression may cement himself as the most dominant group member, thus settling the dominance contest. We only occasionally observed lower-ranking males to fight back during FCA. It appears then, that more high-ranking males, and especially the alpha males, play an important role in determining how females receive new males. Selecting alpha males who may be capable of aggressively fending off FCA may be a pivotal decision for those seeking to conduct multi-male rhesus introductions. Many individual differences among these males could influence their likely response to this challenge; personality factors, past social history, age, weight and dominance style (i.e., males who establish their dominance through displays near the females may be more likely to receive aggression than males who take a more passive approach) are possible factors and should be investigated further (Bastian et al., 2002; Bercovitch et al., 2003; Capitano, 1999; Drickamer and Vessey, 1973; Kempes et al., 2008; McCowan et al., 2011; Rox et al., 2018, 2019). It would also be interesting to more fully investigate the responses of lower-ranking males, but overall, our findings highlight the strong influence of alpha males on the outcome of introduction processes.

4.3. Female Factors Contributing to FCA and Introduction Success

Individual females who lived in larger groups were more likely to participate in FCA. Females from smaller groups participated in FCA events once every 40 h, while females from larger groups participated nearly twice as often, about once every 22 h. Females in larger groups may react to incoming males in this more emboldened manner because of the larger number of familiar, possible coalitionary partners in their groups. Previous studies of both wild and captive rhesus macaques have demonstrated higher rates of within group competition in larger groups (Balasubramaniam et al., 2014), or when population density increases (only adult females engaged in more aggression) (Judge and de Waal, 1997). The larger female groups also received larger numbers of males during the introductions because we are attempting to achieve more balanced adult sex ratios in our groups (Beisner et al., 2012). We considered whether the size of the incoming group of males may have been a reason they attacked males more often, but there was no correlation between male group size and FCA rate. So, it is more likely that the strength of female relationships in large groups may account for the finding. It is somewhat surprising that the number of offspring a female had in her group, the number of days each group spent without males before the introduction, as well as the size of her matriline were not related to the likelihood of her being involved in FCA events. Therefore, these FCA events do not appear to be necessarily kin-based coalitions. However, since the FCA had, on average, almost half the matrilines in a group, mutualism and rank maintenance (Chapais, 1992) may better explain this finding. When multi-matriline coalitions aggress incoming males, all participants share the benefits of “evicting” the males and maintaining their dominance rankings. As described by Chapais (1992, page 47), “Females compete for rank as conditional opportunists. They are opportunist in that they are prompt to use any type of advantage in alliance power against any target, not only lower-born ones, and their opportunism is conditional upon their receiving adequate support against the target.”
Older females were more likely to participate in FCA. The rates indicate participation in FCA events once every 50 h for younger females (minimum 3 yrs) and once every 13.3 h for older females (up to 22 yrs). This approximately four-fold difference associated with age is important for colony managers to be aware of. This is consistent with the observation that younger females may be more afraid of these new adult males (Bernstein and Ehardt, 1985). Contact and non-contact aggression is more frequent as a function of age in female rhesus macaques, and older females are more likely to participate in prolonged agonistic encounters than any other age-sex class (Bernstein and Ehardt, 1985). Older females had typically experienced numerous male introductions throughout their adult lives and therefore were possibly more bold during the familiar process. This finding seems to differ with the result of similar work reported by Rox et al. (2018) in which older females with more experience with male introductions were faster to tolerate the new males. They reported that female age was the main factor influencing female tolerance of males. We did not directly measure female tolerance of males, but we will report further on how the male introduction affected social interactions within social groups in future publications. One major difference between the current study and that by Rox et al. (2018) is that only a single male was introduced in their groups which could certainly alter tolerance and aggression by females. It would also be interesting to investigate whether female reproductive state (e.g., pregnant, lactating) and age of youngest infant impacts their response to males, which we were unable to do with this dataset.

4.4. FCA Severity Index

The average severity rating of FCA events was 6.46 out of twelve, and most of them had a moderate level of aggression. Those groups of males who received the attacks with higher severity ratings were less likely to be successfully introduced to the females, substantiating our hypothesis. This is not surprising as such severe interactions would have been factored into decisions about whether males should be permanently removed from the females, hence contributing to an introduction failure. This shows the strength of the female group in preventing male entrance in some cases. We found that in successful introductions, aggressive behavior from individual females within a coalitionary event never caused physical trauma (e.g., bites, injuries). In contrast, there were at least three instances of physical trauma to the males resulting from FCA in unsuccessful introductions. Although we found no relationship between male group size and the frequency of FCA, male group size and severity of the attacks were correlated. Larger male groups received more severe FCA. The severity of FCA did not reduce over time as the introduction proceeded, nor were there differences in severity over the course of introductions for either the successfully or unsuccessfully introduced groups.

5. Conclusions

Captive female rhesus macaques form a tightly-knit group of related and unrelated individuals that support each other via jointly-directed aggression toward new males during their introductions. This FCA sometimes prevents new males from integrating into a group, which can have profound impacts in a captive setting. Unsuccessful male introductions during the relatively short rhesus breeding season often lead to wounding trauma for both
males and females, and if no males are integrated, to a lack of reproduction for that female group for an entire year. Female groups without males can sometimes become unstable without the benefits policing males provide, also contributing to more trauma (Beisner and McCowan, 2013; Flack et al., 2006). Therefore, FCA and female behavior during male introductions should be studied in more depth, as this will provide valuable information to colony managers.

Specific recommendations based on our findings for those wanting to form breeding groups of rhesus macaques are (1) Expect coalitions of females to aggress new males; (2) Monitor larger groups and those with older females for additional time, as they may be particularly likely to aggress new males, or reduce the size of these female groups to diminish this outcome; (3) Expect higher-ranked males to be the target of more female aggression; (4) Select alpha males who may be capable of fighting aggressive females to help complete introductions successfully; and (5) Consider introduction procedures that would better mimic the wild behavioral pattern of migration.

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References


Fig. 1.
Female Age and FCA Rate. The age of females was positively associated with their level of involvement in female coalitionary aggression directed to adult males.
Fig. 2. Female Group Size and Mean FCA Rate. The size of the female group was positively associated with the frequency of female coalitionary aggression directed to adult males.
Fig. 3.
Severity Indices and Introduction. Those male groups that were successfully introduced to groups of females received female coalitionary aggression that was less severe than those whose introductions failed.
Fig. 4.
Severity Index Sum and Male Group Size. Male groups of larger sizes received more severe female coalitionary aggression.
Fig. 5.
Alpha Males’ Responses to FCA and Introduction. Groups with alpha males who responded to more FCA events with aggression were more likely to be successfully introduced than those with other alpha males.
<table>
<thead>
<tr>
<th>Female Group</th>
<th>Intro Year</th>
<th>Female Group Size</th>
<th>Adult Females (≥3 years)</th>
<th>Number of Matrilines</th>
<th>Male Group</th>
<th>Male Group Size</th>
<th>Total FCA Events</th>
<th>Average Severity Rating</th>
<th>Success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2017</td>
<td>34</td>
<td>14</td>
<td>11</td>
<td>A</td>
<td>3</td>
<td>10</td>
<td>7.2</td>
<td>Yes</td>
</tr>
<tr>
<td>Group 2</td>
<td>2017</td>
<td>94</td>
<td>39</td>
<td>6</td>
<td>B</td>
<td>7</td>
<td>33</td>
<td>6.2</td>
<td>No</td>
</tr>
<tr>
<td>Group 3</td>
<td>2017</td>
<td>57</td>
<td>26</td>
<td>5</td>
<td>C</td>
<td>5</td>
<td>14</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>Group 4</td>
<td>2018</td>
<td>39</td>
<td>14</td>
<td>3</td>
<td>A</td>
<td>3</td>
<td>4</td>
<td>7.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Group 1</td>
<td>2018</td>
<td>41</td>
<td>14</td>
<td>11</td>
<td>D</td>
<td>4</td>
<td>13</td>
<td>6.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Group 5</td>
<td>2018</td>
<td>43</td>
<td>18</td>
<td>5</td>
<td>C</td>
<td>5</td>
<td>10</td>
<td>7.3</td>
<td>No</td>
</tr>
<tr>
<td>Group 6</td>
<td>2019</td>
<td>62</td>
<td>35</td>
<td>4</td>
<td>E</td>
<td>7</td>
<td>19</td>
<td>6.1</td>
<td>No</td>
</tr>
<tr>
<td>Group 3</td>
<td>2019</td>
<td>75</td>
<td>31</td>
<td>6</td>
<td>D</td>
<td>4</td>
<td>11</td>
<td>5.5</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Descriptive information on the eight study groups.
## Table 2
Operational Definitions of Behaviors Collected.

<table>
<thead>
<tr>
<th>Category</th>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Aggression</td>
<td>Open mouth stare</td>
<td>Unwavering gaze with widening of the eyes and mouth open.</td>
</tr>
<tr>
<td></td>
<td>Head bob</td>
<td>Abrupt lowering of the head while staring at another individual</td>
</tr>
<tr>
<td></td>
<td>Brow flash</td>
<td>Staring with exaggerated raising and release of the eyebrows</td>
</tr>
<tr>
<td></td>
<td>Ear flaps</td>
<td>Staring while quickly pulling the ears forward and then releasing them</td>
</tr>
<tr>
<td></td>
<td>Lunge</td>
<td>Jumping quickly at another individual</td>
</tr>
<tr>
<td></td>
<td>Slap</td>
<td>Hitting or striking another individual, contact is brief</td>
</tr>
<tr>
<td></td>
<td>Push</td>
<td>Shoving another individual, with hands, away from their present location. Contact is brief.</td>
</tr>
<tr>
<td></td>
<td>Threat and follow</td>
<td>Vocal threat, such as a huff, or a silent threat while walking after an individual</td>
</tr>
<tr>
<td></td>
<td>Short Chase</td>
<td>Running threateningly after the recipient for less than or equal to six meters</td>
</tr>
<tr>
<td>Moderate Aggression</td>
<td>Long Chase</td>
<td>Running threateningly after the recipient for greater than six meters</td>
</tr>
<tr>
<td></td>
<td>Wrestle/Grapple</td>
<td>Prolonged physical contact with another individual where one animal attempts to restrain or restrict the other’s movement.</td>
</tr>
<tr>
<td></td>
<td>Pin</td>
<td>Holding an animal to the ground for at least 3 s. Can be scored when accompanied with pulling out hair, but score “bite” instead when pinning is accompanied by biting.</td>
</tr>
<tr>
<td>Severe Aggression</td>
<td>Bite</td>
<td>Physical contact involving the teeth.</td>
</tr>
<tr>
<td></td>
<td>Injure</td>
<td>Typically biting aggression that results in puncture, laceration, or crush trauma to the body or ears, tails, or digits</td>
</tr>
<tr>
<td></td>
<td>Direct Aggression</td>
<td>Initial aggressive behavior of a conflict by one monkey toward another monkey</td>
</tr>
<tr>
<td>Aggressive Interaction Type</td>
<td>Redirection</td>
<td>Aggressive behavior or displacement by one monkey toward another monkey in response to an approach or aggression by a third monkey.</td>
</tr>
<tr>
<td></td>
<td>Partial Intervention</td>
<td>Intervener functionally supports one individual/party from the original conflict, but not the other</td>
</tr>
<tr>
<td></td>
<td>Impartial Intervention</td>
<td>Intervener is not functionally supporting one animal/party over the other</td>
</tr>
</tbody>
</table>

Operational definitions of aggressive behaviors collected during observations of male group introductions to female groups.
Table 3

FCA Severity Index.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rating 1</th>
<th>Rating 2</th>
<th>Rating 3</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event duration highest</td>
<td>0 – 60 s Mild (threat, lunge, mild slap, short chase)</td>
<td>61 – 120 s Moderate (long chase, grapple)</td>
<td>&gt;120 s Severe (bite, pin, injury)</td>
<td>60.2 s Moderate</td>
</tr>
<tr>
<td>aggression level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Female group participating</td>
<td>0 – 19 %</td>
<td>20 – 38 %</td>
<td>39 – 57 %</td>
<td>20.24 %</td>
</tr>
<tr>
<td>% total matrilines participating</td>
<td>0 – 33 %</td>
<td>34 – 66 %</td>
<td>67 – 100 %</td>
<td>47.50 %</td>
</tr>
</tbody>
</table>

The severity index developed to characterize aspects of FCA events with the mean score from all FCA events.