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Factors Influencing the Success of Male Introductions into Groups of Female Rhesus Macaques: Introduction Technique, Male Characteristics and Female Behavior

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Abstract

In captive populations of rhesus macaques, novel adult males are commonly introduced to female groups every few years to prevent inbreeding, which mimics male dispersal in wild macaque populations. However, introducing adult males is challenging because macaques are aggressive to newcomers, which can result in serious injuries. Efforts to reduce trauma risk during the introduction process and increase the probability of success are needed. Here we investigate the impact of multiple factors, including male attributes (e.g., age, weight, rank, experience), introduction method (punctuated vs. continual exposure to females), and female behavior, on males' trauma risk and integration success. We studied eight introductions of multimale cohorts (3-7 males each; N=36 total) into existing female groups of rhesus macaques at the Yerkes National Primate Research Center. Four cohorts were introduced using the punctuated exposure method where adult males were moved each morning from run housing to the females' indoor enclosure and returned to run housing in the afternoon, and four cohorts were introduced using the continual exposure method where adult males were moved to an introduction enclosure attached to the females' outdoor compound, allowing males to live in protected contact next to the female group continuously. Generalized linear mixed models fitted to trauma risk (e.g., latency to first trauma; total trauma count) and success or failure to integrate (i.e., continual residence within the female group for >53% of days within a 28-day window after first overnight stay) showed that continual exposure to females in the introduction enclosure reduced male trauma risk and increased the likelihood of successful integration compared to punctuated exposure. Males received less trauma when they received a higher rate of grooming from females. Male attributes had no effect. These findings highlight the importance of introduction technique and female behavior in the process of males' social integration into female groups.

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social behavior; introduction enclosure; male introduction; social management; trauma

Introduction

Rhesus macaques (*Macaca mulatta*) are one of the most common primates used in biomedical research (Carlsson et al., 2004), and at research facilities, breeding colonies of rhesus macaques are frequently housed in naturalistic social groups that resemble wild populations. In free-living rhesus macaques, females remain in their natal groups while males emigrate around puberty which reduces the potential for inbreeding (Berard, 1999; Drickamer & Vessey, 1973; Greenwood, 1980). In captivity, male transfer is mediated by colony managers, as macaques do not avoid consanguineous inbreeding with patrilineal kin (although they do avoid breeding with matrilineal kin) (Smith, 1995). A common strategy is to introduce novel adult males (removing resident males prior to their introduction) to groups of females every few years to prevent males from mating with their daughters (Bailey, Young, et al., 2021; Rox et al., 2021). A potential added benefit of introducing novel adult males is improved social stability of the group because adult males are important policers of within-group conflict (Beisner et al., 2012, 2016; McCowan et al., 2011), and adult males that are unrelated to the females are more effective policers than natal males (Beisner et al., 2012; Jackson et al., 2012).

Despite these genetic and social benefits, introducing novel adult males into macaque groups is risky and the outcome unpredictable. In multiple macaque species, immigrant males attempting to enter an existing group may receive aggression from resident group members, and in both captive and wild settings this can result in severe injuries or unsuccessful immigration, (Bercovitch, 1997; Bernstein & Mason, 1963; Lindburg, 1969; Packer & Pusey, 1979; Rose et al., 1972; van Noordwijk & van Schaik, 1985) and potentially even in death (van Noordwijk & van Schaik, 2001; Wilson & Boelkins, 1970; Zhao, 1994). Similarly, captive group formations in macaques, in which unfamiliar animals are either added to a small nuclear group or simultaneously introduced to each other, are also characterized by high levels of aggression and increased risk of injury, including trauma rates nearly four times higher for adult males rhesus compared to adult female rhesus in one case (Bernstein & Mason, 1963; Westergaard et al., 1999).

When introducing novel adult males to captive groups, colony managers aim to minimize the risk of serious injury to the males and increase the chances of males' integration within the social group. Indeed, males' receipt of trauma during the introduction process may be associated with their chances of long-term integration because intense aggression toward newcomers abates as males become familiar with all group members and establish clear social relationships with them (Bernstein, 1964; Bernstein & Mason, 1963; Rose et al., 1972; Rox et al., 2019; Westergaard et al., 1999). Continued receipt of high levels of aggression throughout the introduction process may indicate that some females do not tolerate the male and that he has not attained a stable position within the group (Rox et al., 2018). To successfully integrate novel adult males into female groups with minimal risk of injury, a greater understanding of the factors influencing the outcomes of male introductions is necessary.

The literature on male-male competition, female mate choice, and reproductive success points to several factors that may influence males' social integration into a new female group. As in many primate species (Cowlishaw & Dunbar, 1991), male mating success in rhesus macaques is often (though not always) correlated with dominance rank (Berard, 1999; Bercovitch, 1997), which might suggest that male dominance rank, or traits that aid in rank acquisition (e.g., large body size, prime age (not geriatric or subadult)), may be preferred by females and/or facilitate males' entry into a new social group. However, in species such as rhesus and Japanese macaques where males often enter a new group at the bottom of the hierarchy and there is little aggressive competition for rank or access to estrous females (Berard, 1999; Bercovitch, 1997), we might also expect such traits to have little bearing on males' entry into a group. Sexual selection theory predicts that females should be selective in their mate choice. Among macaques, there is some evidence that females may prefer to mate with dominant males (Manson, 1992; Massen et al., 2012; Rebout et al., 2017; but see Soltis et al., 1999), novel males (Manson, 1995), and males that have a long-term grooming relationship with females (Massen et al., 2012).

Comparatively less research has examined the factors influencing males' successful entry into a new group, particularly male introductions in captivity. In wild and free-ranging populations of rhesus macaques, males immigrate during the breeding season (Drickamer & Vessey, 1973; Lindburg, 1969), and natal dispersal occurs around 4-6 years of age (Colvin, 1986; Drickamer & Vessey, 1973), indicating that male age/ sexual maturity and the timing with respect to female sexual receptivity influence male immigration success. Indeed, full grown prime age males are more successful at entering the group at high-rank than subadult and older males (Boelkins & Wilson, 1972; Sprague, 1992; Suzuki et al., 1998). A recent study of single-male introductions into captive rhesus macaque groups reported greater success with prime age males and with groups containing more adult females. Further, males were more likely to obtain a long-term stable position in the group when they were heavier, lived in their natal group for longer (i.e., > 3.5 years), and when introduced to female groups with fewer matrilines and no pregnant females (Rox et al., 2019). However, in only 59% of these single-male introductions did males become socially integrated (Rox et al., 2019), underscoring that much remains unknown about influential factors. The introduction of multi-male groups is likely to be more complex than single male introductions, because each male must negotiate his relationship with females and the other incoming males. Female sexual receptivity facilitates immigrant males' entry into the group because of females' interest in potential mates. However, female interest will vary across males, so their behaviors toward immigrant males in multi-male groups may further complicate integration of the males.

Another potential source of influence is the introduction process itself. In a recent publication, we describe a new method for introducing multiple adult males to female groups which allows continual exposure to the females by housing males in an introduction enclosure attached to the female compound. We compared this approach with a method of punctuated exposure (i.e., moving the males from run housing to the indoor enclosure attached to the female compound, and returning them to run housing each day). For multi-male introductions in particular, the introduction enclosure increased the relative success of multi-male introductions, with success defined as maintaining a sex ratio of at least 1:10 was maintained for 28 consecutive days (Bailey, Young, et al., 2021).

Here we present a more thorough examination of the impact of these introduction enclosures as well as male attributes and female behavior, on male introductions. Specifically, we examined male receipt of trauma during the introduction process and each male's progress toward successful integration into the female group, as measured by the proportion of days spent overnight with the female group in the 28-day window following the first overnight. Since staff members decided each day whether males would stay overnight with females or not, this reflected staff confidence in group safety. We hypothesized that males having continual exposure to the females during the introduction would be less likely to receive trauma during the process, compared to males with punctuated exposure, and more likely to socially integrate with the females. This is because continual exposure to the females prior to the physical introduction allows males to get better acquainted with the females and their social dynamics. We also tested the influence of males' attributes (e.g., age, weight, and dominance rank), predicting that prime age, heavier, and/or high-ranking males would be less likely to receive trauma, and more likely to stay overnight with females than others, as past research suggests that these males are more successful immigrants (Boelkins & Wilson, 1972; Sprague, 1992) and may be preferred by females (Massen et al., 2012; Rebout et al., 2017). We also expected that prior experience living in a breeding group as an adult would impact trauma risk and number of overnights with females. Finally, we examined the relationship between females' behavior toward the males and our proxies of success because female-initiated social interactions likely reflect females' interest in and acceptance of the males and how well the process of social integration is going (Rox et al., 2018).

Methods

Ethics Statement

The Yerkes National Primate Research Center (YNPRC) facility and all its associated programs are AAALAC International accredited. All animal procedures were approved by the Emory University IACUC, adhered to the American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates, and 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.

Study Subjects and Groups

Study subjects were rhesus macaques living at the YNPRC Field Station. Across three breeding seasons (September to December) from 2017-2019, five cohorts of adult and subadult male rhesus macaques (Tables 1 and 2; three cohorts used twice) were introduced to six unique groups of females (Table 1; two groups used twice). Female breeding groups lived in outdoor compounds (size range: 5,265 – 10,000 sq. ft.), each with access to an indoor area divided into two spaces: a smaller 'capture unit' (to separate or access individual animals) connected to a larger living space (size range: 286 – 359 sq. ft.). All animals had unrestricted access to fresh drinking water and commercial monkey diet, and enrichment was routinely provided including fresh produce, climbing structures, foraging devices, and manipulanda.

The choice of study groups, and assignment of a male cohort to each study group, was made collaboratively between Colony Management, Colony Genetics, and the research team. A subset of suitable multi-male groups was chosen for each female group based upon genetic compatibility (genetically dissimilar), the male group that would most improve the adult sex ratio, and/or included males with the most prior breeding experience.

Male Introduction Procedure

The introduction of one or more non-natal, adult males is conducted every 3-4 years for each female breeding group at YNPRC Field Station as part of standard colony management procedures. All male introductions proceeded according to existing colony management protocols, with input from the research team. Colony managers decided the approximate timing of each planned male introduction to coincide with female estrous, based on timing of the previous season's births. Resident males were removed 1-48 days prior. For the traditional or "punctuated exposure" introduction method, the males were moved in the morning from their run housing to part of the indoor enclosure attached to the females' compound for 5-7 hours/day and were returned to run housing each afternoon. During the day, females could enter an adjacent indoor area to see the males and have limited physical contact through chain-link fencing. As positive interactions (e.g., proximity and grooming) increased between males and females and negative interactions decreased (e.g., contact aggression), colony managers released the males into the outdoor compound with the females. If positive interactions continued, they gradually increased the duration males spent in the compound daily. Males stayed overnight with the females for the first time when staff were able to leave the group unattended for several hours during the day without severe conflict or injury. Colony managers maintained their standard practices of monitoring and observation of male introductions throughout the study. All management decisions were based upon behavior and made by colony managers in consultation with research staff.

Under the "continual exposure" approach, the males were moved from run housing to an introduction enclosure connected to the outdoor portion of the females' compound and remained there, as the introduction enclosures were equipped for permanent housing (e.g., wind-break panels and heaters (Bailey, Young, et al., 2021)). Males had continual visual access to the females and protected contact through chain-link fencing. As with punctuated exposure, interactions between the males and females were monitored by research staff and colony management staff. As positive interactions increased, males were released into the outdoor compound with the females. Periods of full access were monitored and lengthened over time, and eventually males were allowed to stay overnight with the females.

When serious injury or concerning aggression was observed, one or more males were temporarily removed (e.g., for veterinary treatment). Males were sometimes removed as a unit (and the introduction was paused) and sometimes removed individually while other males in the cohort remained, depending on the male's rank and introduction history. For example, if a high-ranking male was removed, his entire cohort was removed to prevent inter-male aggression and rank shifts that could lead to further injury. If a lower-ranking male was removed, the introduction continued for the other males. Finally, for cohorts with evidence of male incompatibility during the current or previous year's introduction, males were treated individually for any necessary removals, because risk of inter-male aggression was no greater than if males were removed as a unit.

Behavioral Data Collection

A team of two to three observers conducted behavioral observations throughout the breeding season for each introduction. All observers achieved a reliability score of 100% agreement in animal identification and 95% agreement on all behaviors, which were calculated using Krippendorff's alpha (Krippendorff, 2011). Observations occurred from 8am – 3pm Monday through Thursday during the breeding season, barring days when the introduction was paused (e.g., inclement weather, or injury to a high-ranked male that required removal for treatment). During visual/ protected contact, observers noted the males' behavior (i.e., interactions with other males and with females through the caging); data collection on female-female interactions within the compound was not conducted during this phase. Once males were released into the female compound, behavioral interactions were recorded among all group members 3 years of age and older.

Using an event sampling design, one observer recorded aggressive (e.g., threats, chases) interactions and a second observer recorded status signaling (e.g., displacements, silent-bared-teeth signals) interactions. Observers recorded the participants' identity and their behaviors/ responses. Complex, polyadic events such as interventions by a third party or redirected aggression were recorded as an ordered series of pairwise interactions connected via a common label in the data. These observation methods have been successfully applied in past work on social stability in large captive groups of rhesus macaques (Beisner et al., 2011; McCowan et al., 2011).

Affiliative interactions, including social grooming, huddling/ social contact, and proximity (sitting within arm's reach), were recorded using scan sampling. During observation hours, an affiliation scan was performed every 20 minutes.

Male Attributes and Calculation of Behavioral Variables

Males' weight (in kilograms), age (in years), and the number of months of prior experience living in a breeding group as adults were obtained from colony records. Males' weights were measured 1-4 weeks before the start of their introduction. Regarding prior experience, the median number of months living in a breeding group as an adult was 22.2 (range: 0 – 60 months). For five of the males (3 in cohort Wk and 2 in cohort Fe) their first introduction to a female group (since leaving their natal group) was examined during this study. Relative dominance ranks among males (ordinal and categorical: high/mid/low) were calculated from submissive interactions among males while housed in bachelor groups in indoor-outdoor runs during the 2-4 weeks (50 hours total) prior to the start of the introduction. Males' ranks did not change during the first three weeks of full contact with females and remained the same throughout the introduction for most cohorts. However, in three of the eight introductions, a rank shift occurred after a male was removed for treatment of trauma.

To summarize females' behavior toward the males during the introduction process, we calculated females' aggression toward each male, grooming initiated with each male, and sexual behaviors (e.g., solicitations, rump presents, consorts/pair sitting) directed toward each male. We calculated each male's rate of receipt of these behaviors in two ways: (1) the total frequency of each behavior directed toward the male, per female per hour of observation, and (2) the number of unique females directing each behavior toward the male, per female per hour of observation. We focused on behavioral interactions recorded once males were released into the females' compound (rather than interactions recorded during visual/ protected contact) because our outcome variables (i.e., trauma and number of overnights spent with females) require males have full contact with the females. Because the patterning and duration of males' releases into the females' compound varied widely across groups, we summarized all behavioral variables for two different time frames: (i) the entire duration of the introduction, from the date of first release into the females' compound until 3 weeks past first day spent overnight with females or until the introduction failed (male's total hours with females: mean = 284.9 hrs, range 20.5 – 738.3 hrs), and (ii) the first 16 times (or unique days) each male was released into the females' compound (total hours with females: mean = 97.1 hrs, range 20.5 – 340.8 hrs). Although most introductions required more than 16 releases to the female compound prior to their first overnight stay with females, for rapidly progressing introductions with fewer than 16 releases before staying overnight, the 16 unique days included days prior to overnight stay and days after.

Trauma Data Collection

We documented all significant trauma received by males, from females or other males, during the introduction process and throughout the breeding season. Significant trauma was defined as that requiring temporary removal from the group for veterinary treatment. When possible, males brought to the hospital for veterinary treatment were returned to their group the same day to minimize time away from the group.

The eight male introductions varied in duration, pace, and pattern (Supplemental Information: Tables S1–S8). We generated three outcome variables from the data on trauma to measure trauma risk in a consistent manner across introductions despite the considerable variation in the progression of each. (1) The summed duration of time (in hours) a male was released to the females prior to the first instance of significant trauma received; for males that never received trauma, this duration was the total hours present in the females' compound during the entire introduction study. (2) The total count of significant trauma received per male during the first 16 release attempts into the female compound. There was a maximum of one release attempt per day, and some days (e.g., weekends/ holidays) the males were not released to the females. For introductions where males rapidly integrated with the females and the total number of releases prior to staying overnight were few (e.g., male cohort Fe began staying overnight with the females of group IV on the third day), we counted trauma during the first 16 *days* in the female compound. We chose the first 16 releases/ days because this time frame encompassed a significant proportion (i.e., greater than 33%), of the introduction phase, and represented a standardized time frame to compare across groups that captured variation in receipt of trauma. (3) The total count of significant trauma received per male throughout the entire introduction process. The end of the introduction was defined as either 3 weeks past the first day spent overnight with females or the date the introduction was declared failed and aborted by Colony Management staff.

Overnight Stays with Females

Males stayed overnight with the females for the first time when they had been left unattended for several hours during the day without issue. After this first night, males remained with the females 24 hours per day unless they received or dispatched concerning levels of aggression and/or significant trauma. As an additional measure of introduction progress or success, we counted the total number of days that each male spent overnight with the females during the 28-day time frame following the males' first night with the females. Males that stayed overnight with females for a greater number of days during this time period were more successful than those that stayed overnight for few days. The 28-day time frame is based upon our recently published definition of introduction success i.e., maintaining a male:female sex ratio of at least 1:10 for 28 consecutive days and nights (Bailey, Young, et al., 2021).

Statistical Analyses

To examine males' risk of receiving trauma during their introduction to a breeding group of females, we performed three sets of analyses. In the first analysis, we conducted a survival analysis of the time (in hours) until each male first received severe trauma during the introduction process using a mixed-effects Cox proportional hazards model (N=38). If a male never received trauma during the introduction and these observations were right-censored in the survival analysis. In the second and third analyses, we fitted a generalized linear mixed effects model (GLMM) to the count of traumas per male during the first 16 releases/ days with the females and the count of traumas per male during the entire introduction process, respectively. A negative binomial family distribution was necessary for the second, and third analyses due to overdispersion in the counts of trauma, and an offset variable was included for the total hours spent with the females. Finally, we performed a fourth analysis of the count of overnight stays in the female compound per male during a 28-day time window after their first overnight stay. The count of overnight stays showed a bimodal distribution, with values clustering at or near zero and at or near 28 days (Supplementary Information, Figure S1), so we transformed the count of overnight stays into a binary outcome: successful introduction (the male spent >53% of the 28 days overnight with females) and failed introduction (the male spent <28% of days overnight). No animals were in the 28-53% range of days overnight. We analyzed this outcome measure using a GLMM with a binomial family distribution.

For all analyses, we included either cohort identification (ID) or male ID as random effects (whichever term most improved model fit) because three cohorts (N=12 males total) were studied in two different introductions, resulting in repeated measures for these males/ cohorts. Fixed effects were male attributes (weight, dominance rank, age, and months of prior experience in breeding groups), introduction method (punctuated vs. continual exposure to females) as well as the number of days the female group was without males prior to the introduction start, and females' behavior toward the males during the first 16 days of the introduction (total grooming given to males, number of unique females that groomed the males, total initiation of sexual behaviors with males, unique females initiating sexual behaviors with males, total aggression given to males, unique females directing aggression at the males). All analyses were performed in RStudio (R version 3.6.1) with the following packages: *coxme* (Therneau, 2020) and *glmmTMB* (Brooks et al., 2017).

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

I. Descriptive statistics of the male introductions

Four of the male introductions were successful (3 continual exposure, 1 punctuated exposure), one was partially successful as one of the five males became socially integrated, and three were unsuccessful (Table 1). At the individual level, there were a few males whose cohorts failed to integrate, but who individually were able to live with the females for nearly 28 consecutive days and nights (see Supplemental Information Tables S1–S8). Thus, individual level success differed somewhat from cohort level success.

II. Survival analysis of time until first trauma

There were two best-fit models of time until first trauma (Model 1 AIC=87.7, Model 2 AIC=89.3, dAIC=1.6; Table 2), and both were better than the empty model with only a random effect for male ID (AIC=103.8). According to these models, the risk of receiving trauma was 77.4% – 82.3% lower (hazard ratios: 0.226 and 0.177) for males with continual exposure to females compared to males introduced with punctuated exposure (Table 2, Figure 1). Model 2 also showed a nonsignificant trend that trauma risk may be greater for males who received more aggression per day during their time in the females' enclosure (Table 2).

III. Analysis of the count of trauma during first 16 releases/ days in female group

There were two best-fit models of the count of trauma males received during their first 16 releases/ days in the female group (Model 1 AIC=78.6, Model 2 AIC=80.4, dAIC=1.8; Table 3), and both were better fit than the empty model with only a random effect for male ID (AIC=92.8). These models showed that males who received a higher rate of grooming from females received less severe trauma during the first 16 releases to the female group than males who were groomed less (Table 3; Figure 2).

IV. Analysis of total trauma received during the introduction

There were two best-fit models of total severe trauma received by each male during the entire introduction (Model 1 AIC=115.7, Model 2 AIC=117.2, dAIC=2.8; Table 4), and both were better fit than the empty model with only a random effect for male cohort ID (AIC=132.2, dAIC=6.4). According to both models, males received less total trauma when they received a higher rate of grooming from females (Table 4). In addition, total trauma received was 4.1 times lower among males with continual exposure to the females in the introduction enclosure compared to punctuated exposure (Table 4: Model 1).

V. Analysis of successful vs. failed introduction via overnight stays in the female group

There were four best-fit models of male successful vs. failed introduction (Model 1 AIC=39.3, Model 2 AIC=39.7, Model 3 AIC=40.1, Model 4 AIC=40.5; Table 5), and all were better fit than the empty model with only a random effect for male cohort ID (AIC=44.4). Models 1 and 2 included nonsignificant terms for rate of grooming received from females and rate of aggression received from females, respectively. Models 2 and 4 both suggested that males were more likely to be successfully introduced with continual exposure to females in the introduction enclosure than with punctuated exposure (Table 5; Figure 3). Finally, Model 4 indicated that males were more likely to be successfully introduced when the introduction began shortly after removal of resident males.

Discussion

Introduction of novel adult males into female groups is a common practice for captive breeding colonies of rhesus macaques which mimics the natural pattern of male migration in wild populations. Rhesus macaques are often aggressive toward newcomers (Bernstein & Mason, 1963), and introducing unfamiliar adult males to a group of females is risky and often unsuccessful (Bailey, Young, et al., 2021; Rox et al., 2019). To reduce the risk of injury and improve the likelihood of males' successful integration, we developed a new introduction method: an introduction enclosure attached to the females' outdoor compound that allows multimale groups to live continuously next to the females. Across all analyses, the primary predictors of trauma risk and introduction outcome (measured by proportion of 28 days the male stayed overnight with females) were introduction method and female-initiated behavior toward males. Surprisingly, male attributes and prior experience as breeding adult males had no effect.

All three analyses of trauma showed that trauma risk to the males was lower with continual exposure to the females. Survival analysis showed trauma risk was 77.4% – 82.3% lower, and total trauma received was also much lower when introduction enclosures were used. Similarly, males succeeded in staying overnight with the females for the majority of the 28-day period when an introduction enclosure was used. This bolsters our recently published finding that using the introduction enclosure increases the chance of successful social integration for multi-male cohorts compared to punctuated exposure (Bailey, Young, et al., 2021). Both low risk of trauma and the ability to live continuously with females are important measures of a male's level of social integration. Resident animals' initial aggressive response toward immigrant males typically abates within the first few hours to days of contact (Bernstein, 1964; Bernstein et al., 1977; Bernstein & Mason, 1963; Rox et al., 2019), whereas continued high levels of aggression (and trauma risk) may indicate that some females do not tolerate the male and that he has not attained a stable position in the group (Bailey, Bloomsmith, et al., 2021; Rox et al., 2018).

We suspect that the reason for lower trauma risk and greater success in living continuously with females is the greater visual access and protected contact with the females that introduction enclosures offer compared to only 5-7 hours with the punctuated exposure method. Males in the introduction enclosure also can view a wider range of female behavior in the outdoor compound, whereas males housed in the indoor area, are limited in the social information they can obtain. Protected contact is a common first step when introducing unfamiliar animals (e.g., pair housing) and is intended to reduce the risk of injury while allowing animals to become familiar and begin to establish a dominance and/or affiliative relationship (DiVincenti & Wyatt, 2011; Truelove et al., 2017). The extended visual access and protected contact provided by the introduction enclosure may have allowed males to gain key social knowledge to facilitate establishing relationships with the females and navigating their new social environment (e.g., discern the female hierarchy) and may better approximate the process of male group transfer in wild and free-ranging populations, which can span several weeks and often begins with brief visits to the new group (Drickamer & Vessey, 1973; Lindburg, 1969).

How recently the resident males were removed prior to the start of the introduction of new males also predicted introduction success – males introduced shortly after removal of resident males were more likely to successfully integrate with females than those introduced over a month after. Conventional thought is that females will more readily accept new males if the previous males have been gone for longer, and our results contradict this notion. However, multiple factors (e.g., female social dynamics as well as logistical issues such as weather or short staffing) contribute to how quickly new males are introduced following removal of resident males. Some female groups that responded to the removal of their resident males with increased aggression (e.g., Groups III and VI, Table 1) received new males more rapidly (with the intention of preventing social unrest). Future studies should examine the length of time female groups are without males as well as the potential causes or correlates of this, such as female group dynamics.

Our results also showed that grooming received from females predicted males' receipt of trauma (i.e., trauma counts, but not latency to first trauma) – males that received a higher rate of female grooming had lower trauma risk, supporting the notion that greater receipt of grooming from females is a behavioral indicator of females' acceptance of the male (Rox et al., 2018). This is also consistent with previous findings that females prefer to mate with males with whom they have an established grooming relationship (Massen et al., 2012). Interestingly, we found female sexual interest in males (i.e., receipt of female-initiated sexual behaviors) was not associated with trauma risk or overnight stays, despite the fact that male immigration in wild and free-ranging groups occurs during mating season (Boelkins & Wilson, 1972; Drickamer & Vessey, 1973; Lindburg, 1969) and the presence of pregnant (vs. estrous) females can negatively impact male introduction success (Rox et al., 2019). However, because grooming also often occurs between male and female pairs that are mating or in consort (Hill, 1987), it may be that our measurements of grooming behavior captured both female sexual interest as well as their social acceptance of males. We also found a nonsignificant trend that aggression received from females was associated with greater trauma risk in the analysis of time until first significant trauma. Although social aggression is the cause of trauma, the lack of a clear relationship between aggression received and trauma risk has been documented before in captive groups of macaques (Beisner et al., 2019; Judge et al., 1994; Ruehlmann et al., 1988).

Surprisingly, male attributes such as weight, age, rank within the multimale group, and prior experience as breeding adult males did not significantly predict trauma risk or success in staying overnight with females. This is in contrast to the findings of Rox and colleagues (2019) that prime age and heavier males were more successful in introductions in captive rhesus macaque groups, and findings from wild and free-ranging populations that prime age males are more successful at entering at high-rank than subadult and older males (Boelkins & Wilson, 1972; Sprague, 1992; Suzuki et al., 1998). A couple of potential reasons may explain this. First, the ages of the males in this study skewed young. Of the 26 unique males studied, 23 were under 8 years of age at the time of introduction, the approximate age when male rhesus achieve full body size (Bercovitch & Goy, 1990). The other three males were prime age (i.e., 9-15 years old; see Table 1) and had already achieved full adult size at the time of introduction. Age was also confounded with high rank in our study subjects, as the oldest males were also the highest ranked in their cohorts. Thus, there may not have been sufficient variation in male age, size, and rank to be able to see their effects. Age was also correlated with males' experience because young males have little to no previous experience living as an adult in female groups. Rox and colleagues (2019) were able to address this confound between age and experience by analyzing their data with and without young males. However, young males predominate our data set. Future studies should examine cohorts of older males to better examine the influence of experience, age, weight, and dominance rank.

Another potential reason for the lack of influence of male attributes was our focus on multi-male introductions, involving 3-7 males per cohort, unlike (Rox et al., 2019) who examined single-male introductions. The influence of male age and weight may be easier to detect in single-male introductions due to the increased social complexity of multi-male introductions which require males to not only build relationships with the females but also continue to navigate their relationships with the other males. Females are presented with more choices in multi-male than in single-male introductions. While females' preference for one male over another may facilitate his entry into the group, these preferences may also be based on additional male attributes (e.g., temperament). A final consideration is that the trauma received by the introduced males was frequently caused by the other males in their cohort, such as lacerations and puncture wounds, whereas male-inflicted wounds are not possible for single-male introductions to captive female groups because resident males are often removed beforehand (Rox et al., 2018, 2019). Of the 47 total records of trauma to the males, 30 involved a laceration or puncture wound. Thus, our outcome variable of trauma received may be less applicable to single-male introductions or contexts when resident males are absent or have been removed.

Our results suggest that colony managers conducting male introductions would benefit from allowing males to live next to the female group with continual visual access and protected contact. Enclosures that allow continual exposure to females can reduce staff effort as well as stress to both staff and animals because males do not need to be moved back and forth between run housing and the female compound each morning and afternoon (Bailey, Young, et al., 2021). In addition, the reduced incidence of trauma to males with continual exposure improves animal welfare and further reduces staff effort needed to access, transport, and treat injured animals. Our results also suggest that behavioral observations to monitor the amount of grooming the males receive will also yield valuable information about males' likely trauma risk and chances for social integration. Since one of our analyses (trauma received during first 16 releases) showed that the number of *unique* females that groom a male may provide just as much information as total rate of grooming received, it is possible that behavioral observations need only focus on the number of unique female partners rather than total frequencies of grooming received, which would make these observations more practical to conduct.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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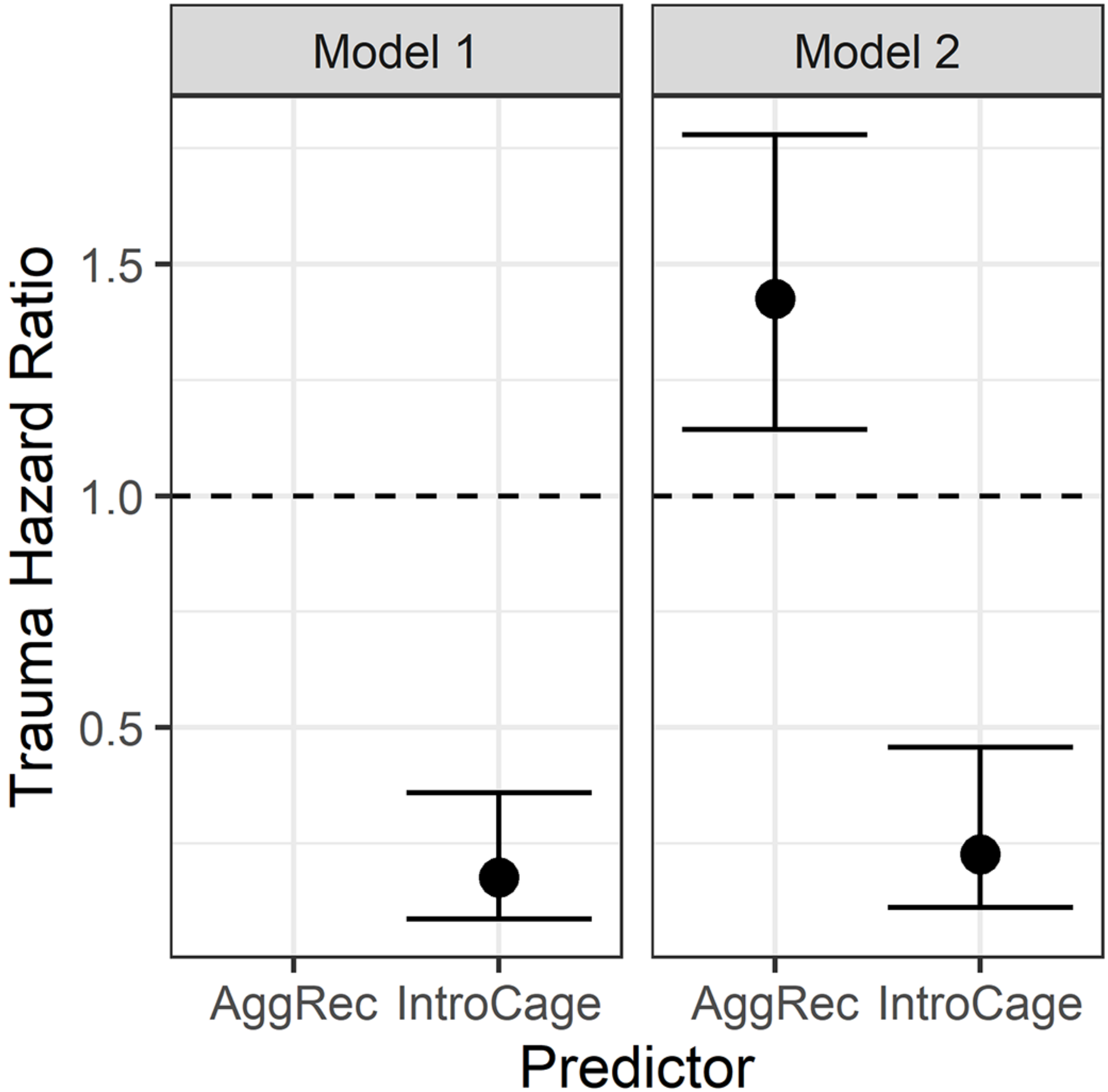

Figure. 1.
Hazard ratio (*e*^coefficient) with 95% confidence intervals from the survival analysis of time to first severe trauma for: the introduction enclosure, which allows continual exposure (relative to punctuated exposure) (Models 1 & 2) and aggression received from females per hour of observation (Model 2).

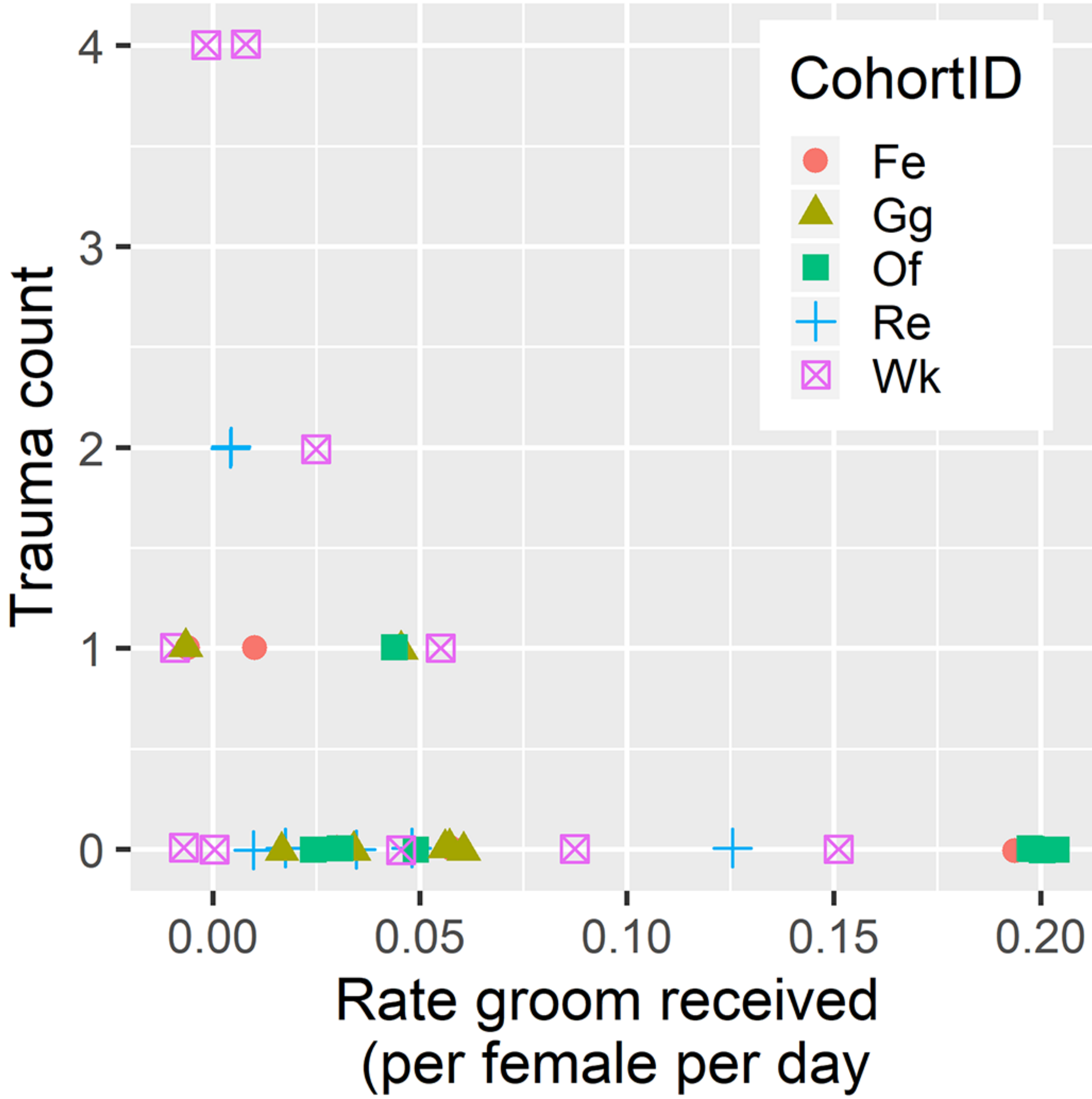

Figure 2.

Count of trauma received by each male during the first 16 releases/ days in the female group plotted against the rate of grooming males received per female per day.

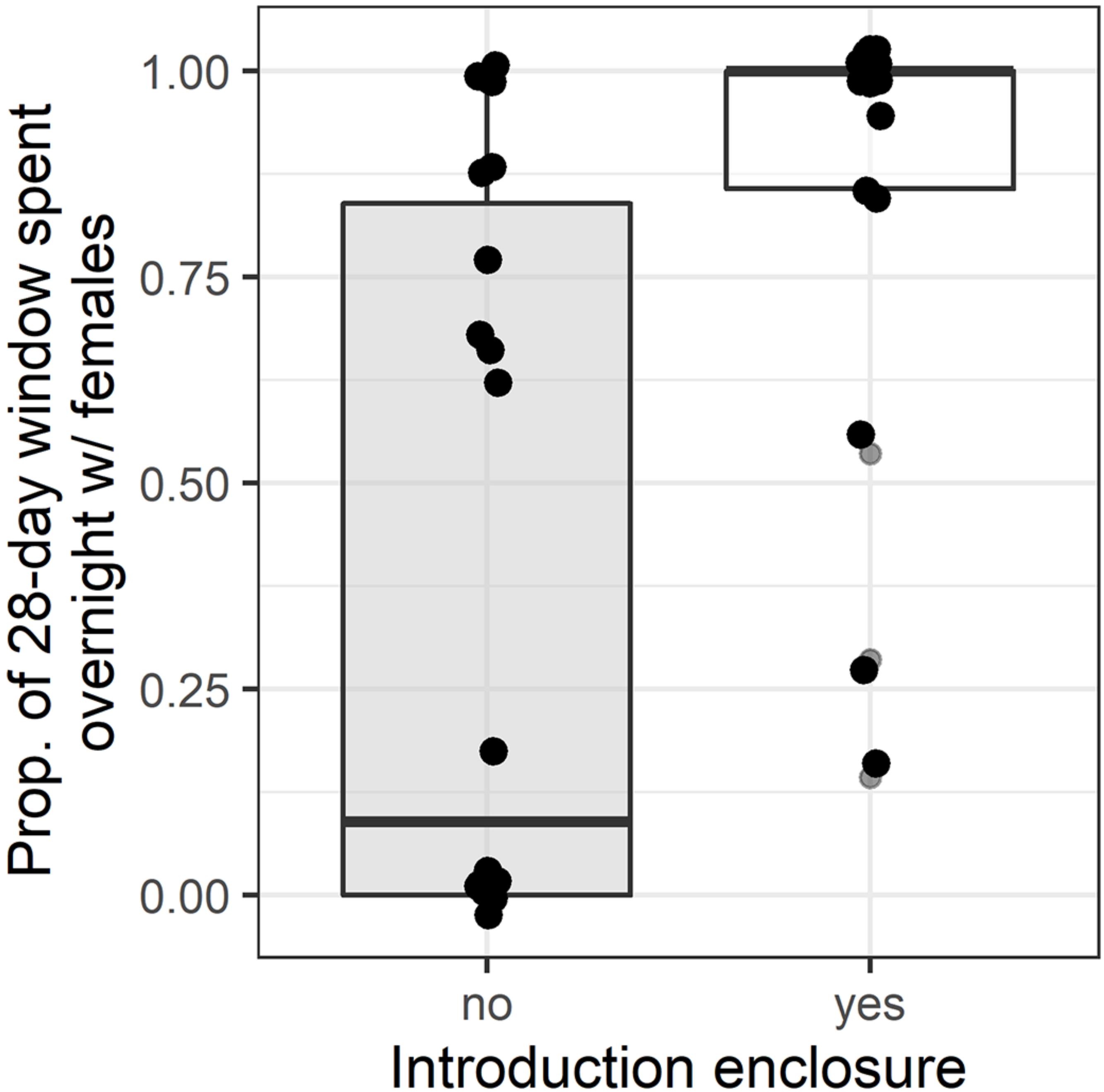

Figure 3.
Boxplot, with overlaid scatter plot, of the proportion of the 28-day window each male spent overnight with the females for those introduced using the introduction enclosure (white box) and those introduced using the standard method (gray box). The upper and lower ends of the box represent the upper and lower quartiles.

Table 1.

Characteristics of female breeding groups and the male cohorts introduced to them

Year	Female group	# Adult females (group size)	Male cohort ID	Males ages	Introduction method	Introduction Outcome
2017	I	40 (84)	Re (N=7)	7, 6, 6, 6, 6, 6, 6	punctuated exposure	fail
2017	II	26 (64)	Wk (N=5)	10, 9, 5, 5, 5	punctuated exposure	fail
2017	III	14 (33)	Fe (N=3)	14, 5, 5	punctuated exposure	success
2018	IV	14 (33)	Of (N=4)	6, 6, 6, 6	continual exposure	success
2018	V	14 (41)	Fe (N=3)	15, 6, 6	continual exposure	success
2018	VI	18 (44)	Wk (N=5)	11, 10, 6, 6, 6	continual exposure	1 male successful
2019	VII	37 (60)	Gg (N=7)	7, 7, 7, 7, 7, 7, 7	punctuated exposure	fail
2019	II	32 (76)	Of (N=4)	7, 7, 7, 7	continual exposure	success

Table 2.

Model output for the two best-fit models in the survival analysis of time until first significant trauma

	Predictor	Coefficient	Hazard Ratio	SE of Coefficient	P-value
Model 1 AIC=87.7	Intro Enclosure (yes vs. no)	−1.734	0.177	0.71	0.014
Model 2 AIC=89.3	Intro Enclosure (yes vs. no)	−1.488	0.226	0.704	0.035
	Aggression received per day (unique females)	0.355	1.426	0.221	0.11

Table 3.

Output for the two best-fit models in the analysis of trauma received during the first 16 releases/ days with the female group

	Predictor	Coefficient	SE	P-value
Model 1 AIC=78.6	Grooming received per female per day (unique females)	−47.06	15.34	0.002
	Intro enclosure (yes vs. no)	−1.55	0.68	0.024
Model 2 AIC=80.4	Total grooming received per female per day	−16.00	7.72	0.038

Table 4.

Output for the two best-fit models in the analysis of total severe trauma received during the introduction

	Predictor	Coefficient	SE	P-value
Model 1 AIC=115.7	Total grooming received per female per day	−6.38	2.25	0.004
	Intro enclosure (yes vs. no)	−1.42	0.61	0.020
Model 2 AIC=118.5	Total grooming received per female per day	−1.081	0.235	<0.001

Table 5.

Model output for the two best-fit models in the analysis of each male's introduction success or failure, as measured by the proportion of overnight stays with the females during the 28 days after the first overnight stay

	Predictor	Coefficient	SE	P-value
Model 1 AIC=39.3	Total grooming received per female per day	8.89	6.36	0.16
Model 2 AIC=39.7	Intro enclosure (yes vs. no)	2.96	1.61	0.066
	Aggression received per female per day	−20.85	15.65	0.18
Model 3 AIC=40.1	Number of days without males	−0.07	0.03	0.043
Mode 4 AIC=40.5	Intro enclosure (yes vs. no)	2.77	1.41	0.049