Sex Differences in Hierarchical Stability in a Formation of a Mixed-sex Group of Rhesus Macaques

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Forming groups of captive rhesus macaques *(Macaca mulatta)* is a common management practice. New formations of unfamiliar macaques can be costly, with high levels of trauma, particularly as intense aggression is used to establish a dominance hierarchy. Combining previous subgroups into one new group may be beneficial, as some individuals already have established dominance relationships. We tested this hypothesis by forming a new mixed-sex group of rhesus macaques that combined an established group of females with an established group of males. Prior to the mixed-sex group formation, both the female and male hierarchies had been stable for 3 y; after mixed-sex group formation these hierarchies were maintained by the females and were initially maintained by the males for 3 wks. However, the temporary hospitalization (due to a laceration caused by aggression) of the alpha male destabilized the male hierarchy. Age and weight then predicted male rank. Temporary hospitalizations resulted in rank changes for the males, evidenced by reversals in subordination signals. This study indicates that using established groups of familiar individuals may maintain female hierarchical stability in a mixed-sex group formation, but further research is needed to understand how to maintain and predict male hierarchical stability to reduce trauma. Improved knowledge of hierarchical stability would be invaluable to managers of large rhesus macaque groups and would help improve the welfare of captive rhesus macaques.

Abbreviations: NHP, non-human primates; **pSBT**, peaceful silent-bared teeth display

Social group formations of captive nonhuman primates (NHPs) occur frequently for a variety of reasons (for example, breeding, research purposes, permanent housing arrangements). Rhesus macaques are the most used NHP in biomedical research.[15] Classified as highly despotic, rhesus macaques rely on a heavy use of aggression to maintain and reinforce their dominance hierarchy,[47] which ultimately governs access to resources and mates.[50] Extensive aggression can result in significant traumas and even death.[12,51] Reducing trauma is therefore an important goal both to maintain the animals' welfare and to minimize the associated cost of care. Thus, this research is aimed at improving the success of forming groups with minimal trauma.

One strategy to potentially improve the success of group formations and decrease aggression is to mix familiar individuals with an already established dominance relationship.[18,53] However, dominance ranks are not static and can change if aspects of the social environment change,[1,11,17] such as the loss of keystone individuals[34] or addition of new allies.[17] As many NHPs are housed in pairs or small social groups, substantial research has been aimed at understanding pairing success,[14,31,38] but less is known about factors that contribute to successful formations of large, naturalistic social groups. Because high levels of aggression and trauma can occur even in well-established groups,[8,45] gauging social stability in large group formations of rhesus macaques is difficult, as aggression does not necessarily equate to incompatibility or trauma.[8,31,38] Understanding whether individuals maintain their previous hierarchies is critical information for behavioral managers as they form new social groups.

Wild female rhesus macaques remain in their natal groups and acquire adjacent ranks to their mothers through coalitionary support.[10] Males also retain their ranks near their mothers while in their natal groups,[16] but after dispersal at sexual maturity and entry into a new breeding group, males mainly enter the group at the bottom of the hierarchy and move up after dispersals or deaths (see[26] for general background information and an unusual case of rank acquisition). Because males emigrate from multiple natal groups, many of them will be unfamiliar to one another. Whether male dominance relationships in their natal groups affect their rank in subsequent groups as adults is uncertain, although some research suggests that postnatal nepotism occurs in long-tailed macaques *(Macaca fascicularis)*, with males maintaining a high dominance rank longer in non-natal groups when other male kin are present.[25] In many groups in which males originate from multiple natal groups, tenure in the new social group often dictates male dominance rank.[26]

The current research sought to determine whether female and male rhesus macaques would maintain their established same-sex hierarchies during a group formation in which an established group of females was introduced to an established group of males. If previous relationships are maintained, this

could indicate that using established groups of familiar same-sex individuals could lessen the trauma and associated costs of a group formation, as individuals would not need to use high levels of aggression to sort out their hierarchy. We predicted that previous social rank, rather than individual attributes, would predict social rank in the new mixed-sex social group.

Materials and Methods

Feeding and IACUC approval. Subjects in all portions of the study were fed commercial monkey chow (LabDiet 5047 High Protein Monkey Diet Jumbo, Hayward, CA.) and a scratch mixture of seeds and foraging enrichment twice daily. Water was provided ad libitum. Supplemental produce (fruits and vegetables) was provided once or twice weekly. All procedures adhered to the NIH Guide for Animals and were approved by the UC Animal Care and Use Committee.

Female natal group hierarchical stability. One social group of rhesus macaques was studied from March 2017 to May 2019 at the California National Primate Research Center (CNPRC) in Davis, CA. Subjects were part of a multigenerational mixed-sex social group formed in 2003. Across the 2 y period, a total of 129 adults (3+ years in age: 88 females; 41 males) comprised the group, although the number varied as individuals were permanently removed from or aged into the adult population by turning 3 y of age. Macaques ranged in age from 3 to 22 y. All subjects lived in a 0.20 ha outdoor enclosure with multiple climbing structures and visual barriers (for example, hanging barrels, culverts, and pole perches).

The group was studied during 5 different time periods, 4 of which were 16 wk in duration (March to June 2017, 2018, and August to December 2017, 2018) and one of which occurred for 8 continuous weeks (March to May 2019). In each study period, data were collected 6 h per day for 4 d per week. One observer recorded all aggressive interactions using an event sampling strategy. The identity of the initiator and recipient macaques was recorded, as was the intensity of aggression (mild: threats, short chases of less than 6 m; moderate: grapple, long chase of 6m or more; and severe: biting/pinning). Any third parties that intervened were also recorded. A second observer used an event sampling design to record status interactions, defined as unprompted submissive interactions (such as from a simple approach). Status interactions included silent-bared teeth display (SBT), present rump, freeze/turn away, move away, and run away. Interobserver reliabilities ranged from 0.85 to 1.0 (Krippendorff's α).

For the purposes of studying the longitudinal stability of the female hierarchy in their natal group, only interactions among the adult females (excluding interactions with adult males) were analyzed to focus on the female hierarchy. A total of 56,543 female-female dominance interactions (aggression and status combined) were recorded (spring 2017: 13,739; fall 2017: 11,166; spring 2018: 13,694; fall 2018: 10,829; spring 2019: 7,115).

Bachelor group hierarchical stability. In July 2016, 14 males (ranging in age from 2.5 to 5 y old) were removed from their natal group at the CNPRC. Males were placed in a corncrib structure (2 round sections of 4.42 m in diameter by 1.83m high connected by a 2.44m wide × 3.05m deep by 1.83m high anteroom) and resided there until they were relocated to another corncrib structure (2.44 m high × 14.63m wide × 7.32m deep) in 2019, which they resided in until September 2019. Two males were permanently removed from the group for health and social reasons.

As part of routine monitoring, behavioral management staff recorded hierarchical displacements 1 to 2 times per month (from July 2016 to September 2019) in 15 to 20 min sessions and constructed quarterly dominance hierarchies. Data points were entered into a win/loss matrix in excel. Animal identities were listed on both axes, with the initiator of the displacement on the Y axis and the retreating animal on the X axis. The matrix was then re-ordered manually to find the best rank order, which was defined as the order that had the majority of the displacements in the top diagonal of the matrix (this area represented interactions that were consistent with the current hierarchy) and as few displacement points as possible below the diagonal (this area represented interactions that were contrary to the current hierarchy). Inter-observer reliabilities were conducted semi-annually, with a criterion of Krippendorff's α greater than 0.80.

Mixed-sex group formation and data collection. In September 2019, the 12 adult males, ranging from 5 to 8 y of age, were placed in a novel outdoor, 0.20 ha field enclosure. One week later, adult females were introduced. The number of adult females introduced was guided by previous studies on optimal sex ratios in both captive and wild populations.[5] The introduction was made during the annual mating season (from approximately August to February) to increase the likelihood that females would accept the novel males. Over a 3-wk period, 1 to 4 adult females per day were introduced into the male enclosure, along with male and female juveniles (1 to 2 y old). Younger females were prioritized for selection from the natal group to introduce to the novel males, as prime aged females are likely more receptive to novel males.[41] The alpha female and the beta matriline were not introduced into the new social group for age and social reasons, so key females were missing. Females were also generally introduced in order of dominance rank, although not in strict ordinal rank. After the third week, the alpha male of the group was temporarily hospitalized due to a shoulder laceration, which ended all further introductions due to male hierarchical instability. At the end of the 3-wk period, 27 of the 67 adult females that were in the natal group in 2019 (age range: 3-19 y, average age, 7.5 y), 12 adult males, 19 juveniles (12 females), and 12 infants (5 females) resided in the new group. If this new mixed-sex group maintained previous dominance relationships, only the intersex dyads would have to establish dominance relationships (324 dyads: 12 males * 27 females; not analyzed in this study), as compared with 1,521 dyads if all individuals had been unfamiliar with one another (39 individuals * 39 individuals).

During the first month, the group was monitored for 12 h per day, 7 d per week, by 2 observers (the same individuals that observed the female natal group). After that, the group was monitored for 6 h per day (from 0900 to 1200 and 1300 to 1600), 4 d per week (Monday, Tuesday, Thursday, and Friday) for 15 continuous weeks. During this time, data were collected using the same protocol as in the female natal group. A total of 3,118 female-female dominance interactions (aggression and status) and 1,439 male-male dominance interactions were observed.

Dominance hierarchies. Elo-rating[35] was used to calculate dominance ranks in all population segments (female group stability and male group formation), except for the bachelor group. Elo-ratings are numerical representations of dominance ranks, with higher values reflecting higher dominance ranks. Elo-ratings are continuously updated based on wins and losses and the expected outcome, with more points attributed to unexpected outcomes (that is, a lower-ranking individual exhibiting aggression toward a higher-ranking individual). Two Elo-ratings are generated for each interaction: one for the winner and one for the loser. Given the continuous updating, Elo-ratings are ideal for tracking rank changes over time.[35,53,54] The *elo.sequence* function[35] was used in R software (version 3.3.3, R Foundation for Statistical Computing, Vienna, Austria).

Each individual started at an initial value of 1,000 and a k value of 200. Mean Elo-ratings were used for analyses.

Demographic information. Weights (in kg) were recorded at biannual health exams by veterinarians, which occurred immediately before the group formation. Age was determined through birth records. Also, given that males received traumas that resulted in temporary hospitalizations, the number of days a male spent in the hospital (recorded through veterinary records) was included as a variable, as temporary absences have been shown to affect dominance relationships.[37,54] During hospitalizations, males were completely removed from the group (typically for a week), with no visual or auditory access to the social group (but with visual and auditory access to other hospitalized animals).

Dyadic dominance certainty. In addition to calculating dominance ranks, we also wanted to establish whether male-male dyads had *certainty* in their dominance relationships. Dominance certainty exhibits an L- or U-shaped relationship to dominance rank,[42,49] in which individuals of high and low rank exhibit the highest certainty while those of middle rank exhibit the least certainty and considerable variation.[49] To calculate dominance certainty, we used the Percolation and Conductance method[22,24] in R software (version 3.3.3) on all male-male dominance interactions (status and aggression). This method uses both direct and indirect pathways to construct a network, while gauging an animals' overall 'fit' or 'certainty' within the network.[42,49] Greater consistency in the direction of pathways results in greater certainty, whereas greater inconsistency in the flow of pathways results in less certainty.[22,24,49]

Association between variables and dominance rank. Due to the small sample size of adult males (n = 12, 9, and 8 over time; resulting from permanent removals), we used Spearman correlations to test the associations between weight (in kg), age (in years), days spent in the hospital, dominance certainty, matrilineal rank (ordinal rank: 1 to 10) and dominance rank (Elo-ratings) in the mixed-sex social group. Spearman correlations were used to test stability of ranks over time. Pearson correlations were used for the females.

Statistical analyses were conducted in SPSS (version 26; IBM Corp, Armonk, NY) and R software (version 3.3.3). To visualize instability, peaceful silent-bared teeth display (pSBT) networks were constructed using Cytoscape (version 3.7.1, Cytoscape Consortium, Institute of Systems Biology), as social stability is demonstrated by unidirectional and transitive pSBT networks,[23] which may become bidirectional and intransitive during social instability.

Results

Female hierarchical stability in natal group. While residing in their natal groups, the female hierarchy was highly stable over time (see Figure 1), as females inherited their maternal rank (spring 2017 and fall 2017: r = 0.96, R^2 = 0.93, $P < 0.01$; fall 2017 and spring 2018: r = 0.97, R^2 = 0.93, $P < 0.01$, spring 2018 and fall 2018: r = 0.94, R^2 = 0.88, $P < 0.01$). Given that the spring 2019 study was only 8-wk long, whereas the other studies were 16-wk, we only analyzed whether the most recent dominance ranks (in spring 2019) predicted female dominance ranks in the mixed-sex group formation.

Male hierarchical stability postnatally. Only matrilineal rank in the natal group predicted male dominance rank in the bachelor group (2016: r_s = 0.82, $P < 0.01$; 2017: r_s = 0.81, $P < 0.01$; 2018: r_s = 0.88, $P < 0.01$; 2019: r_s = 0.88, $P < 0.01$), which was stable over time (2016 to 2017: r_s = 0.99, $P < 0.01$; 2017 to 2018: r_s = 0.95, $P < 0.01$; 2018 to 2019: r_s = 0.99, $P < 0.01$). Age (2 to 5 y of age) and weight (3.5 to 9.07 kg) played no significant role in dominance ranks postnatally. Thus, maternal rank had long-lasting effects in adolescent and adult male macaque dominance relationships, even in the absence of female kin.

Mixed-sex group formation: male hierarchical instability. After the formation of the mixed-sex group , the male hierarchy remained stable for approximately the first 3 wk, as the ordinal rank in the bachelor group predicted their Elo-ratings in the new mixed-sex group (r_s = −0.99, $P < 0.01$; in this case a lower number (for example, #1) for the ordinal rank indicates a higher dominance rank and a higher Elo-rating reflects a higher dominance rank, thus creating a negative correlation), revealing that the males initially retained their dominance ranks in this new social context. Furthermore, male-male dyads (n = 66 dyads for the first 5 wk of the study) that were more different in matrilineal rank (but not age or weight) in their natal group had greater dominance certainty (r_s = 0.38, $P < 0.01$) in their dominance relationships in the mixed-sex group. However, 20 d after the initial introduction, the alpha male was hospitalized for a large shoulder laceration. Most male traumas happened during the night, making it difficult to determine the identity of the aggressor and the context of the aggressive interaction. Although clearly male-patterned trauma, the injured male did not appear to fall in dominance rank, as all males continued to submit to him before he was removed to the hospital. Four other males had been briefly hospitalized before the alpha male, all of whom were very low-ranking. No rank changes occurred before or after these 4 males were returned to the group. Ranks changes increased only after the hospitalization of the alpha male. During the absence of the alpha male, significant male-male traumas occurred, especially at the top of the hierarchy, resulting in the majority of the males being hospitalized. This situation resulted in a cascade of male rank changes and instability that persisted for the remainder of the study (see Figure 2). This instability was evident not only in male fights but also in reversals in the pSBT network, which are usually perfectly unidirectional and transitive in stable groups.[7,19] While highly
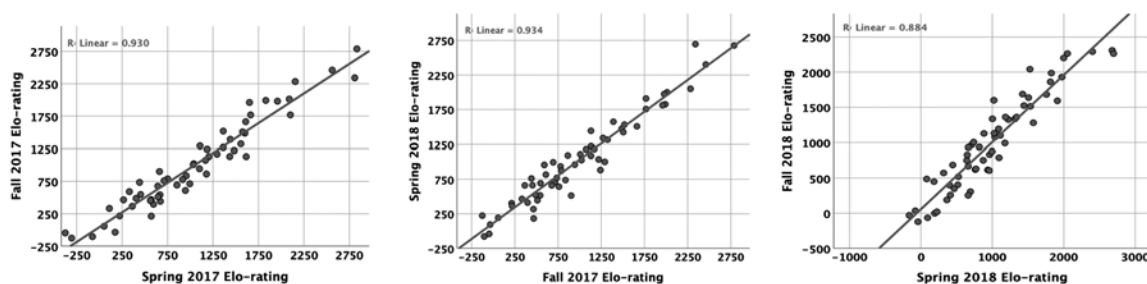

Figure 1. Female hierarchical stability in their natal group over a 2-y period. While residing in their natal group, the female hierarchy remained stable across the 2 y, reflected by correlations between their previous and current Elo-ratings.

structured in the beginning of the study, the male pSBT network became bidirectional and lacked the complexity (see Figure 3) and consistency typically present in these networks. To allow characterization of changing levels of instability, the 15-wk study was divided into three 5-wk periods.

The results for the middle period (the second 5-wk period, after permanent removal of 3 males; $n = 9$ males remaining) are contrary to the beginning of the formation, as both age and weight, rather than matrilineal rank, predicted male dominance rank (age: $r_s = 0.75$, $P = 0.02$; weight: $r_s = 0.76$, $P = 0.02$). Although age and weight were not significantly correlated ($r_s = 0.53$, $P = 0.08$), determining which predictor is more meaningful is difficult, so both were included. Days spent in the hospital marginally predicted male dominance rank ($r_s = -0.66$, $P = 0.05$), indicating that males that were hospitalized more often had lower dominance ranks. Furthermore, male-male dyads ($n = 36$; given that 9 males remained in the 2nd 5 wk) that had a significant difference in age ($r_s = 0.40$, $P = 0.02$) and a marginal difference in weight ($r_s = 0.32$, $P = 0.06$) had greater certainty in their dominance relationships as compared with male dyads that were more similar in these physical characteristics. During the middle period, the alpha male was deposed by the beta male (the 10th ranking male prior to group formation) and removed from the group.
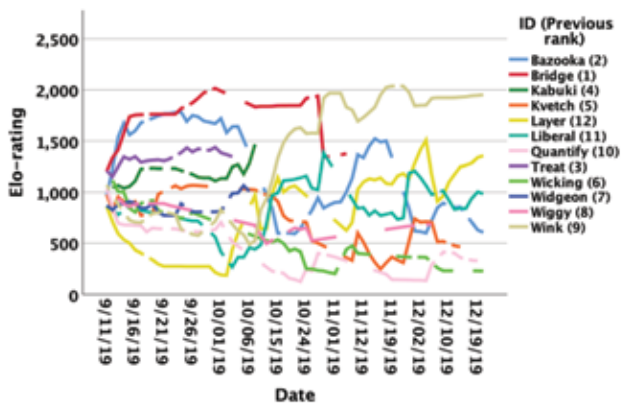

Figure 2. Male rank changes during the mixed-sex group formation. Male dominance ranks changed throughout the 15-wk of the mixed-sex group formation, evidenced by inclines and declines of their Elo-ratings.

The results for the final period after group formation (the third 5-wk period, 8 males remaining) were identical to the middle period, as both age ($r_s = 0.82$, $P = 0.01$) and weight ($r_s = 0.76$, $P = 0.02$) predicted male dominance rank. Similarly, male-male dyads ($n = 29$ given that 8 males were remaining in the last 5 wk) that had a significant difference in weight ($r_s = 0.39$, $P = 0.04$) and a marginal difference in age ($r_s = 0.33$, $P = 0.08$) had greater certainty in their dominance relationship. Days spent in the hospital marginally predicted male dominance rank ($r_s = -0.64$, $P = 0.09$), indicating that males that spent more time in the hospital had lower dominance ranks.

Given the highly dynamic process of male rank acquisition in the group formation, we sought to determine whether hospitalizations resulted in changes in dominance rank or changes in rank resulted in hospitalizations. Because social connections can be formed and strengthened, whereas others can weaken while a male is hospitalized with no visual or auditory access to other group members, we predicted that temporary absences from a dynamic social environment could promote rank changes. We calculated Elo-ratings for all males and transformed this into relative rank (percentage of males currently in the group that each male outranked) on the day a male was hospitalized and then 1 wk after return ($n = 19$ hospitalizations). Relative rank was used instead of Elo-ratings because multiple males were hospitalized at the same time, so males had varying relative ranks at any one time (for example, a male ranking #2 when only 3 males were present is a different situation than ranking #2 when 8 males were present). We then ran a paired-sample t test to examine whether the male that was hospitalized significantly dropped in dominance rank after hospitalization. As predicted, males dropped in dominance rank after hospitalizations ($t_{18} = 2.10$, $P = 0.05$). This can also be seen on Figure 2, on which absences in the line (indicated by a lack of data, usually due to a hospitalization) are followed by sharp declines (that is, drops in dominance rank).

Mixed-sex group formation: persistence of female hierarchical stability. Contrary to the male hierarchy, the female hierarchy ($n = 27$) was consistent throughout the entire 15-wk study (beginning to mid: $r = 0.96$, $P < 0.01$; mid to end: $r = 0.99$, $P < 0.01$), with only their social ranks in 2019 and not age or weight predicting dominance rank (beginning: $r = 0.81$, $P < 0.01$; mid: $r = 0.92$, $P < 0.01$; end: $r = 0.92$, $P < 0.01$). The female pSBT network was complex with multiple layers flowing in a unidirectional path
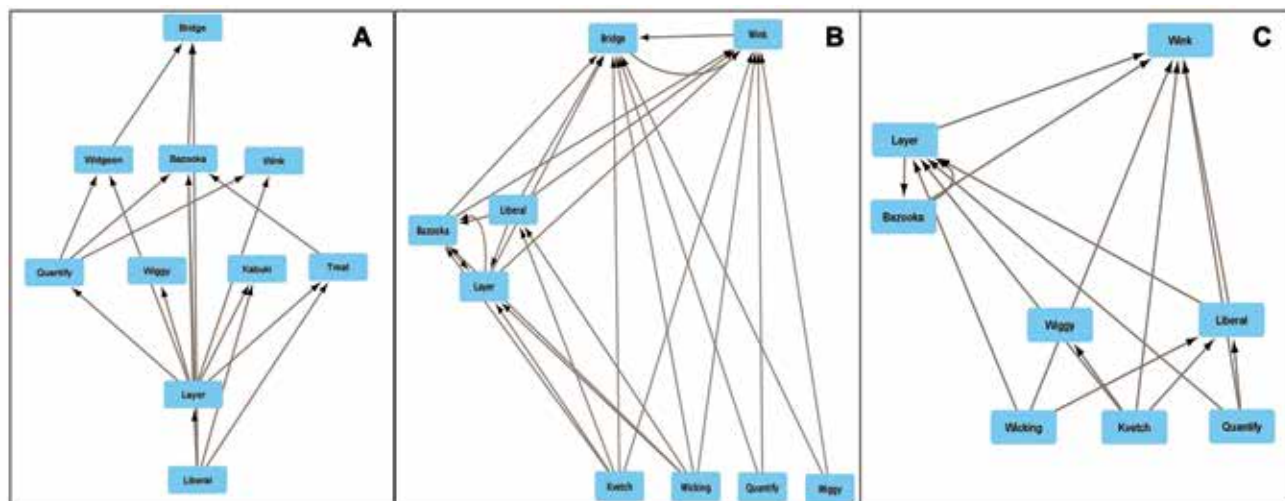

Figure 3. Male pSBT networks across the 15-wk mixed-sex group formation indicate instability. During the first 5-wk (A) of the mixed-sex group formation, the male pSBT network was unidirectional. In the second 5-wk (B), the male pSBT network now had bidirectional edges, reflecting rank changes. In the final 5-wk of the study, the male pSBT network had less bidirectional edges, but still indicated rank changes.
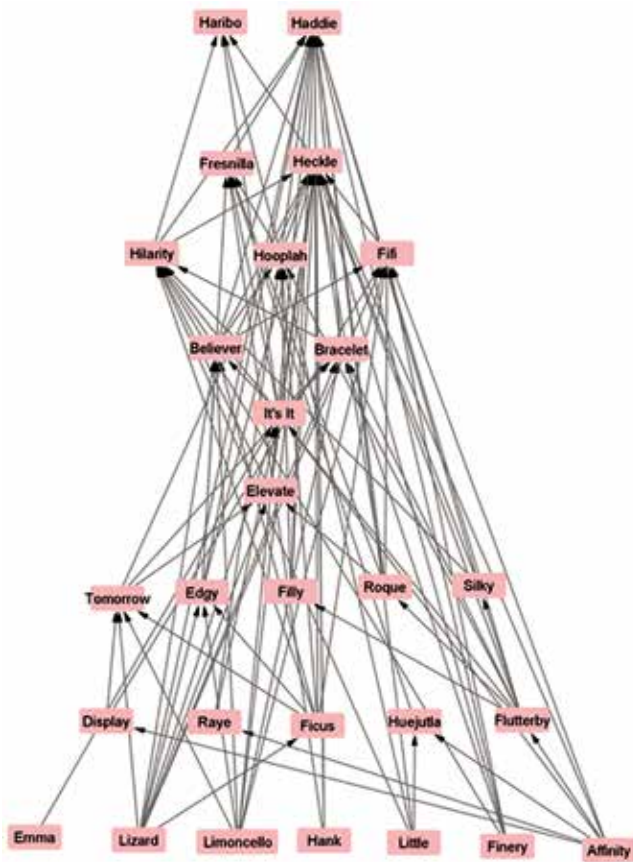
Figure 4. Female pSBT networks across the 15-wk mixed-sex group formation indicate stability. During the entire 15-wk mixed-sex group formation, the female pSBT reflected unidirectional links, with multiple and complex layers all consistently flowing upward, reflecting a stable network.

(see Figure 4), consistent with stable hierarchies[7] and sharply contrasting the male pSBT network. Thus, females retained their established social structure in the presence of new males.

Discussion

In this study, female macaques maintained their sex-specific dominance hierarchy in the presence of novel males,[13] even with the loss of 50% of their female social group. Males retained their hierarchy during the first stage of the mixed-sex group formation, but lost this hierarchical stability after temporary removal of the alpha male due to injury. During the final 10-wk of the study, males challenged each other based on individual attributes rather than relying on previous relationships. These findings indicate that females may be more socially prone to maintain social stability than males, and previous social stability cannot guarantee future social stability for males.

Once removed from their natal groups, the males retained their matrilineal rank while residing in the bachelor group for 3 y. Although males varied in age and weight, dominance rank likely was not achieved through physical means (that is, fighting to establish a dominance hierarchy) in the bachelor group, but by social support (that is, long-term natal alliances). Although maternal rank inheritance has been found to persist in peer groups (that is, in the absence of adult kin) of juvenile males (ages 1 to 3),[30,53] to our knowledge, this is the first study to demonstrate matrilineal stability in adult males (at least housed together in a bachelor group). One possible explanation for the preservation of natal rank is that the costs of fighting were outweighed

by the lack of benefit. While females compete primarily over access to resources such as food,[55] males can compete over mating.[33] Given the lack of females, the risks of fighting may have outweighed any benefits. Indeed, male NHPs can cause significant harm to one another, as their large canines[58] can easily lacerate and puncture deep tissues (for example baboons, *Papio cynocephalus*[20]). As compared with competition for mates, the preservation of dominance rank may alternatively be explained by positive social interactions over time or kinship (as several males were related), as this has been shown to result in less competition (for example, barbary macaques, *Macaca sylvanus*).[9,56]

In addition, pairs of males that were more distant in matrilineal rank (in their natal groups) had greater dominance certainty in their relationships, further indicating that matrilineal rank initially persisted in this new mixed-sex social group. However, instability occurred in the male dominance hierarchy immediately after the temporary removal of the alpha male. Temporary removal of key individuals (both males and females), especially alpha and beta individuals, is known to predict social collapse in rhesus macaques.[6,37,54] Given that high-ranking males (particularly the alpha and beta males) are highly effective at managing and mitigating conflict[21] and controlling trauma,[5] the temporary removal of the alpha male in this study prompted a series of dominance challenges that resulted in excessive male traumas that led to the permanent removal of several males throughout the course of the study (only 7 of 12 males remained on the last day of the study). Thus, once the instability began, physical characteristics became better predictors of dominance rank than were the social factors that had been functioning previously.

Over the middle and end of the mixed-sex group formation (weeks 6 to 14), the main predictors of male dominance rank were age and weight—2 characteristics of physical size and potential fighting ability. In contrast to the bachelor group, the males now had a resource to fight over: fertile females. Although dominant rhesus macaque males do not monopolize females, high-ranking rhesus macaques are generally more reproductively successful.[50,52] Dominant males also have long-term consorts and mate-guard, while also harassing females away from lower-ranking males, resulting in sneak copulations as an alternative tactic for low-ranking males.[36] Thus, males may be motivated to move up in the hierarchy for mating opportunities, and older males are more likely to do so. Furthermore, male dyads with greater differences in age and weight had greater dyadic dominance certainty, indicating that males of different sizes were less likely to challenge each other.

Temporary hospitalizations resulted in lower dominance ranks for males when they were returned. While trauma could be a product of a lower dominance rank, the opposite seemed to be the case. When returned to the group, hospitalized males often experienced aggression from individuals that had been submissive to them before their hospitalization. Given that high-ranking males may inhibit the behavior of subordinate males (for example, vervets, *Chlorocebus pygerythru*[28]), the absence of higher-ranking males may allow other males to form strong bonds with females and build critical alliances and relationships, as female support can be critical to male success.[2,57] The results of our study indicate that during new group formations, providing medical treatment to males without removing them from the group may be advantageous, but if that is not feasible, time away from the group should be minimized to the extent possible to decrease opportunity for new alliance formation. If individuals are potentially problematic in escalating aggression, they should be temporarily removed as a preventative measure. In addition, all reintroductions should be closely monitored.

Like the males, the females maintained their matrilineal rank for the 2.5 y prior to the mixed-sex group formation, although unlike the males, they then remained in their natal groups and had continued access to kin support. Furthermore, unlike the males, during the introduction stages of the mixed-sex group formation, the females lost over 50% of their female hierarchy, including key sources of kin support, resulting in the previous 6th ranked female filling the alpha position in the new group. Matriarchs that can be key to matrilineal stability[4,44] were also removed. Thus, the female hierarchy might have been expected to change, given that matrilines were fragmented[4] and long-term allies were removed. However, the female hierarchy during the mixed-sex group formation remained highly similar to the hierarchy in their natal group and was stable throughout the entire 15-wk of the mixed-sex group formation. While some research suggests that matrilineal fragmentation can promote social instability,[4,44] other research (in elephants, *Loxodonta africana*) suggests that females can rebuild their social networks and maintain stability.[27] However, the group may need instability before for matrilineal fragmentation to escalate social instability. Further, in order to promote social stability, the females were generally introduced to the males in order of dominance rank because the order of introduction can affect rank in rhesus macaques.[43,48] However, even high-ranking females that were introduced near the end of the introduction process were still dominant over females introduced earlier; thus, order of introduction is likely more important in promoting rank changes of unfamiliar individuals. Nevertheless, introduction of individuals in order of established dominance rank is recommended as a potential way to promote rank stability. Indeed, the female hierarchy, unlike the males, remained stable over time, as evidenced by a perfectly unidirectional and transitive network of pSBTS, which signals subordination.

Additional suggestions for managers of captive macaques. Although the established male hierarchy destabilized in the mixed-sex group formation, this does not mean that all mixed cohorts of males will be unsuccessful. For example, this study used males from the same natal group, with ranks "inherited" from their mothers, yet later achieved rank due to physical attributes (e.g., age and weight). Thus, the males did not initially have to sort out their dominance relationships by fighting, but rather simply maintained rank based on years of coalitionary support. Thus, bachelor groups formed from unfamiliar males, which must then establish their dominance hierarchy, likely through fighting, might be more successful in future mixed-sex groups. Indeed, Yerkes National Primate Research Center has formed bachelor groups by using unfamiliar males taken from multiple natal groups, which are usually given a year to stabilize their hierarchy; this approach has generally been successful. Furthermore, the current results suggest that when forming bachelor groups of males, having cohorts of stratified ages and sizes is likely beneficial. The finding that male-male dyads had more certain dominance relationships when they had more distinct differences in size suggests a curb on the likelihood of challenges to the dominance hierarchy, thereby also limiting the possibility of trauma and instability.[14,40] Moreover, prime-aged males that are heavier are also more likely to be successfully integrated into the female group,[39] which can increase stability. Although bachelor groups formed with unfamiliar individuals that are stratified in age and weight are more likely to be stable, previous stability does not guarantee future stability. Therefore, bachelor groups should be continuously monitored for any cases of instability and rank change. Given the intensive nature of continuous monitoring, automated systems to detect potential instability, such as the use of automated feeders[29] or RFID tracking,[32] would provide valuable information and a fruitful area of ongoing and continued research.

Finally, the current process of introducing females into a new group of males, thus giving the males a "home turf" advantage, is counter to how male integrations occur in the wild, where males typically reside on the periphery of the group and slowly enter the female social group (for an example of how to mimic this in captive groups, see[3]). Given that small numbers of males integrate at a time, adding one male at a time, in order of dominance rank, may simulate natural male integration and hence be beneficial. Using a staggered approach drawn out over a longer period of time (for example, months) may promote male social integration. Although time-consuming, this approach may improve the welfare of captive rhesus macaques.

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