



Evidence-based practice

Introducing three new males into a captive lion-tailed macaque (*Macaca silenus*) group

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Keywords: aggression, enclosure design, intragroup interaction, zoo

Article history: Received: 21 Jun 2018 Accepted: 13 Apr 2020 Published online: 31 Jul 2020

Abstract

The introduction of an unfamiliar animal to a new individual or group can be highly stressful for any animal. Various studies have tried to determine the best method for introducing unfamiliar animals to each other, but many disagree on the most appropriate method of introduction. In this study, three new male lion-tailed macaques *Macaca silenus*, were introduced to the resident group in Fota Wildlife Park. The introduction method here was slow, with controlled contact between the new males and resident group over a period of 17 months. Behavioural data were collected using scan and focal sampling to observe the differences in the behaviour of the existing group before and after the introduction of these new males and during the different types of contact ('outside pen', 'indoor house', 'split group' and 'mixed'). Data were analysed using randomisation and a likelihood-ratio G-test. Active behaviours increased in the first 3 months following the introduction of the new males, while self-directed behaviours decreased overall. Aggression was observed most frequently in the first three months after introduction of the new males, during the 'outside pen' period of contact. By September 2018, there was one successful birth within the group, one of the two surviving males was fully integrated into the group, and with the integration of the second male looking promising, overall this indicates a positive introduction and integration.

Background

Breeding programmes, such as the European Endangered Species Programme (EEP), monitor the movement of individuals within and between zoos in order to carefully manage breeding and genetic diversity within captive populations. Forming new social groups and introducing unfamiliar individuals, however, can be dangerous, especially in captivity where space is limited, and high levels of aggression are commonly noted (e.g. in chimpanzees *Pan troglodytes*, Hoff et al. 1996; Brent et al. 1997; in laboratory-housed tufted capuchins *Sapajus apella*, Cooper et al. 2001; in gorillas *Gorilla gorilla*, Jacobs et al. 2014). There are many studies which look at the various methods to introduce new and unfamiliar individuals to a group, with some suggesting slow, careful methods (Hoff et al. 1996; Westergaard et al. 1999; Cooper et al. 2001; Schel et al. 2013), while others noted no difference between sudden or prolonged introduction (Bernstein 1969; Bernstein 1991). Introducing adults to a group has been found to be more problematic than introducing younger (immature) individuals; one study found that, in chimpanzees *Pan troglodytes*, 8% of adult-to-adult introductions resulted in wounding in the introduced or resident individuals, or both, and that wounds were more likely to occur in male-male introductions (Alford et al. 1995). Several studies on primates have shown that maleto-male introductions were more likely to be unsuccessful than

Table 1. The entire study group at Fota Wildlife Park from March 2015 to August 2016.	¹ Removed before the introduction began, ² related males, ³ removed/
died during study.	

Resident Group				New males			
Name	Number	Gender	Date of birth	Name	Number	Gender	Date of birth
Ally	1336	Female	20 Dec 1997	Joe	4759	Male	16 Mar 2003
Во	2726	Female	15 Nov 2011	Quazi	4758	Male	6 Sep 2007 ^{2,3}
Lizzy	3113	Female	2 Mar 2002	Mauzer	4760	Male	1 Jan 2007 ²
Kizzy	3346	Female	14 Nov 2002 ²				
Mogsey	4227	Female	26 Dec 2007				
Tish	4191	Female	28 Dec 2007				
Jade	4356	Female	22 Sep 2009				
Warfy	4444	Female	31 Aug 2010				
Kelly	4467	Female	16 Dec 2010				
Fizzy	4562	Female	26 Sep 2011				
Pat	4603	Female	17 Feb 2012				
Casey	4628	Male	26 Mar 2012				
Oisin	5070	Male	22 Feb 2013				
Hugh	5041	Male	14 Nov 2012				
Sally	5069	Female	2 Dec 2013				
Jamal	4057	Male	23 Mar 1995 ¹				
Ral	4377	Male	4 Apr 2010 ¹				

introductions with females (Alford et al. 1995; Bernstein 1991; Brent et al. 1997; Crockett et al. 1994; Reinhardt et al. 1995).

This paper focuses on the introduction of three new male lion-tailed macaques *Macaca silenus* to the resident group of macaques in Fota Wildlife Park, as recommended by the long-term management plan set out by the EEP. This introduction was slow, with controlled contact between the new males and resident group over the period of this study. In the wild, lion-tailed macaques live in polygynous groups, generally with just one dominant and few other males. Females remain in their natal group, while, upon reaching sexual maturity, lion-tailed macaque males migrate for breeding. Female lion-tailed macaques have generally been reported to prefer unfamiliar males for breeding and are thought to be the least xenophobic among macaques (Kumar et al. 2001). The objectives of this study were: 1. To compare the behaviour of the lion-tailed macaques before and after the introduction of the new males to determine how behaviour had changed; 2. To examine differences in behaviour observed during each type of contact over the course of the introduction; and 3. To evaluate a slow and careful method of introduction.



Figure 1. The lion-tailed macaque (*Macaca silenus*) enclosure at Fota Wildlife Park (right; Fota Wildlife Park, 2018); outside pen attached to macaque house (left; author's own).

Table 2. Description of the types of contact between the female/juvenile group and the new males over the course of the introduction and integration.

Contact type	
Outside pen	Full visual but limited tactile contact between the two groups. Main point of contact was the outside pen attached to the macaque house. This contact type occurred primarily at the beginning of the study.
Indoor house	Full visual but limited tactile contact between the two groups. Points of contact/interaction were inside the macaque house and not visible to the observer.
Split group	The two groups were mixed and split into two different groups again. One group locked inside house; the other group had access to the outside enclosure. The number of individuals in each group varied. Full tactile contact between members of each group. Contact between groups varied day to day.
Mixed	All individuals mixed together with full tactile contact.

Action

Study site and group

This study took place in Fota Wildlife Park, Carrigtwohill, Co. Cork, Ireland. The lion-tailed macaques are housed in a 4200m² enclosure (Figure 1). This enclosure features a large indoor house, divided into several pens, one large enriched area with a visitor viewing window, and a small outdoor pen attached to the house. The outside area is enclosed by a tall wire fence, with gravel substrate near the edge, many trees and bushes in the middle of the enclosure, surrounded by a zone of tall grass. The perimeter of the fence has two glass viewing points for visitors, on either side of the enclosure. The husbandry routine for these macaques remained relatively consistent for the study period, whereby they were fed in the house, as well as by scatter feeds in the outside enclosure. However, the amount of fruit in the diet was reduced significantly from May 2016 onwards.

A detailed description of this group is given in Table 1. Before the introduction of the new males, the group was composed of one dominant male, one subadult male, nine females and six juveniles. After the arrival of the three new males at the zoo, the dominant male (Jamal) and subadult male (Ral) were moved from the group prior to the beginning of the introduction described here. The resident group then was composed of nine mature females and six juveniles. Of the three new males, two of the males were sexually mature adults (Joe and Quazi), and one was a juvenile (Mauzer). The two younger males were paternally related and all three were familiar with one another, as they all came from the same captive group (Belfast Zoo). Over the course of the study, one female (Kizzy) was removed from the group, and one of the new males (Quazi) died. The older male (Joe) was chemically castrated using a deslorelin implant during the study.

Introduction of males

The males were introduced to the enclosure on 31 March 2015. They were given access to the main house's outdoor pen and one indoor pen at first. The integration procedure started with only visual and limited tactile contact between both groups at two points in the enclosure, at the small outdoor pen at the side of the house and inside the house itself. The males and females were moved between both areas frequently, so both groups had regular access to the large outdoor enclosure. There were also periods where the males were mixed with some of the females and juveniles, and periods were both groups were fully mixed. For this article, the periods of contact are defined as: 'outside pen', 'indoor house', 'split group' and 'mixed'. The type of contact for each sampling period was dependent on availability of staff for supervision and the welfare of the macaques (e.g., individuals with injuries were allowed to heal before any further contact). A detailed description of each is given in Table 2.

Data collection and analysis

Data were collected using both instantaneous scan sampling and 15-minute focal sampling for individuals. Data were collected over 2-hr periods, both in the morning (1030 to 1230 h) and in the afternoon (1330 to 1530 h). The macaques were observed only at points outside the enclosure (not inside the house) as to not impact the macaques. For each observation period, the contact type was noted. Data were collected from March 2015 to August 2016, however data collection was not continuous as there were several interruptions in the introduction procedure, such as individuals separated after receiving hormonal implant. Data were also collected over a 2-month period before the introduction of the new males using the same methods. Along with this data collection, observational field notes were also taken, along with keeper observations. All behaviours were grouped by type for analysis and a description of each behaviour is shown in Table 3.

The data were analysed using R 3.4.3 (R Core Team, 2017). For the focal sample data, the mean percentage per day was calculated for each behaviour (n=43). These data were analysed using randomisation tests from the R 'coin' package (Hothorn et al. 2006) to determine if there were any differences in behaviour before and after the three males were introduced. For the scan sample data, the key focal behaviours were affiliative social behaviours, aggressive behaviours and self-directed behaviours. These data were analysed using the likelihood-ratio G-test, which is preferred for analysing activity budgets, as outlined in Bishop et al. (2013). 'No interaction' contact was excluded as it occurred only once in the observation period. The mean percentage of macaques engaged in each behaviour per day was calculated (outside pen: n=13; indoor house: n=12; mixed: n=15; split: n=5). The null hypothesis was that there is no association between these three behaviours and contact type. The standardised residuals (z-score) for each behaviour/contact type were also calculated and used to assess significance; if the value is outside of ±1.96 then P<0.05, if the value is outside of ±2.58 then P<0.01, and if the value is outside of ±3.29 then P<0.001 (Field et al. 2012). Variability is described using standard error (±SE).

Consequences

Changes to behaviour

Mann-Whitney U randomisation equivalent tests were performed on the observed behaviours before and after the introduction of the new males (with 1000 re-randomised pseudosamples) and a statistically significant difference was found in self-directed behaviour (z=-2.09; P=0.03). Self-directed behaviours were observed to decrease after the introduction of the new males, Newman et al.

Table 3. An ethogram of the observable behaviours of the lion-tailed macaques (Macaca silenus) in Fota Wildlife Park during this study period.

Туре	Behaviour	Description
Feeding	Forage	Searching for food
	Feed	Eating of food
	Drink	Drinking of water
Active	Walk	Slow paced locomotion
	Run	Fast locomotion
	Climb	Moving from one area to another in a vertical direction
	Swing	Hanging from an elevated structure
Rest	Sit	Idly sitting
	Rest	Lying down idle
Affiliative	Sexual inspection/presentation	Lifting of the tail area to inspect or smell genital area/presentation of genital area to another
	Mount	One individual climbing on another to initiate copulation/dominance display
	Copulate	Sexual intercourse
	Interaction with infant/parent	Contact between infant and mother, either through vocalisation, playing or carrying
	Allogroom	Individuals grooming or being groomed by another
	Play	Interaction between individuals through chasing, rolling, swinging or wrestling
	Huddle	Two or more individuals sitting close together with no grooming taking place
	Embrace	Individuals embracing one another
Self-directed	Scratch	Individual scratching itself
	Autogroom	Individual grooming itself
Out-of-sight	Out-of-sight	Individual not visible to observer
Pace	Pace	Continuous and repetitive walking back and forth
Vigilance	Vigilance	Straight posture with head scanning or staring

from $1.87\% \pm 0.27$ to $0.71\% \pm 0.21$. A difference was also found in active behaviour before the introduction of the new males and in the three-month period after (z=-1.93; P=0.04). Active behaviours were observed at an average frequency of $7.35\% \pm 1.56$ before the introduction of the new males; this increased to $12.6\% \pm 1.62$ in the 3-month period after.

There was no statistically significant association found between the behaviours analysed and contact type ($X^2(6)=4.35$; P=0.629). The standardised residuals (as described in Field et al. 2012) indicated, however, that aggressive behaviours were observed significantly more frequently than expected during the 'outside pen' period of contact.

Overall behaviour differed little before and after the new males arrived. After the introduction of the new male lion-tailed macaques in Fota, active behaviours increased significantly. This is similar to what was found in previous observations on this group of macaques, after the introduction of a single adult male (Newman et al. in prep). In a captive group of western lowland gorillas, however, Van Weerd et al. (2010) reported that active behaviours decreased after the introduction of a new male. In the present study, it was also found that self-directed behaviours decreased after the new males were introduced. However, as selfdirected behaviours were already quite low overall, this may be of little biological significance.

Aggressive behaviours were found to be significantly higher during the 'outside pen' contact period; this type of contact occurred primarily in the first three months of the introduction of the new males. The introduction of a new adult male into a group can result in aggression, injury and possibly even infanticide (Zaunmair et al. 2015). Studies have shown that in macaques, aggression is higher in the first few moments of introduction of unfamiliar individuals, but tends to decline rapidly after (Bernstein 1964; Clarke et al. 1995; Brent et al. 1997; see also similar results in gorillas, Hoff et al. 1996). This may explain the higher frequencies of aggression during this contact period only; the females were noted to be aggressive towards the male immediately following the introduction, but this level of aggression declined shortly after (personal observation). In general, female lion-tailed macaques have been reported to be the least xenophobic amongst macaques towards new males arriving into a group, preferring them for mating (Kumar et al. 2001). This behaviour is thought to counteract the risk of infanticide (Cooper et al. 2001) and is also seen in other primate species (Hrdy 1979). There was a lot of aggression noted (personal observation, March/April 2015) between the two younger new males and a small number of females, but the younger females were also receptive to the new males, particularly towards the older mature male. One male in particular (Quazi) was more aggressive towards the females

than the other two males and during one of the 'mixed' periods of contact, he was attacked and had to be later euthanised due to his injuries. Although it is unknown what exactly occurred, the older male (Joe) had been observed earlier attacking Quazi, who had been aggressive towards a female (T. Power, personal communication, June 2015).

Aside from this one aggressive male, rates of aggression resulting in serious injuries were generally low within the lion-tailed macaque group in this study, which could be because of the introduction methods, which did not allow for full contact between the two groups. Additionally, at Fota, the oldest male (Joe) was chemically castrated (deslorelin implant). The use of chemical castration is thought to be beneficial in reducing aggression of males in particular, while still allowing for the possibility of future breeding. Vinke et al. (2008) found that chemical castration was more effective at reducing aggression in ferrets *Mustela putorius* than surgical castration and has also been reported to be successful in reducing aggression in lion-tailed macaques (Norton et al. 2000).

In the current study, immediately before the introduction of the three males to the group, the previous dominant male was removed, as part of the long-term management plan for this species. Therefore, it is difficult to determine if the changes in behaviour exhibited by the macaques is due to the absence of the dominant male or the presence of the new males, but both events more than likely played a significant role. Previous studies have shown that the sudden removal, or death, of a dominant male can have negative impacts on social behaviours (Newman et al. in prep; Rhine 1973), often with unpredictable consequences as studies on macaques have shown both increased aggression among females (Oswald and Erwin 1976) and reduced aggression (Singh et al. 1992) following the removal of a dominant male. The presence of more than one male has been noted to have an impact on behaviour also; in two wild groups of lion-tailed macaques, Kumar et al. (2001) reported that the frequency of positive social interaction tended to decrease, between both males and females, and among females, when two adult males were present.

Integration method

A slow method of introduction and integration was used in this study, where the macaques had limited and varied contact with one another over the course of 17 months. As stated earlier, some studies recommend longer methods of introduction. A study by Westergaard et al. (1999) investigated whether sudden (1 day) or prolonged (weeks) introductions impacted on rates of injuries or reproductive success in lab-housed rhesus macaques. The authors found that prolonged introductions, allowing individual introductions, significantly reduces the risk of serious injury in these macaques. Other studies have suggested that a quicker introduction method may be more successful when introducing new individuals. Bernstein (1969) found that the simultaneous introduction of individuals into a group of pig-tailed macaques was far less disruptive and resulted in fewer incidents of aggression than did the slower method of introduction. Furthermore, Bernstein (1991) stated that, based on several studies on introduction procedures with different species of monkey, there is no evidence that gradual introductions were more successful than sudden introductions. He stated that slow introduction periods, or repeated introduction of individuals, can increase agonistic responses. The only exception he stated is when the no-contact period is used to determine compatibility of individuals.

On integrating a proboscis monkey all-male group, Sha et al. (2013) suggested that ideally individuals should be introduced before reaching sexual maturity, individuals should represent a range of ages to facilitate the establishment of dominance, and that enclosure design and feeding techniques (i.e. scatter feeding to reduce competition) are important to successful introductions. The three males introduced in Fota ranged in age from four to 12 years and, as mentioned previously, all three males were familiar with one another, originating from the same captive group, possibly limiting the risk of aggression, which was also found in tufted capuchins (Cooper et al. 2001). The three new males in Fota, at least in the period before mixing, appeared highly tolerant of one another, maintained close physical contact and regularly engaged in grooming (personal observation, April 2015). Although relationships between adult male macaques, especially among lion-tailed macaques, are generally reported as antagonistic (e.g. Singh et al. 2011), there have been several reports indicating positive interactions (grooming and body contact) between adult male long-tailed macaques (e.g. Brent and Veira 2002).

Enclosure design is an important factor to consider in introductions, especially when introducing unfamiliar individuals. It may be necessary for individuals to be able escape from aggressive encounters (e.g. Westergaard et al. 1999); in a study on chimpanzees, Herrelko et al. (2015) suggested that the number of areas available for an individual to move to is more important that the total space. Schel et al. (2013) suggested that a likely reason for integrations being unsuccessful is the spatial constraints in captivity, forcing individuals to interact from the very beginning of the introduction procedure. It should be noted, however, that some studies have indicated that the design of the enclosure did not impact rates of aggression when groups were socially unstable (in pig-tailed macaques, Erwin et al. 1976; in rhesus macaques, Fairbanks et al. 1978). In this study at Fota, the design of the enclosure played an important role. The internal layout of the house and the presence of a smaller outdoor pen provided multiple points of visual and limited tactile contact between the two groups of macaques, possibly preventing major injuries. Indeed, aggression was recorded at its highest during the 'outside pen' periods, where macaques only had visual and limited tactile contact; aggression was observed at low levels during other periods.

Conclusion

The integration of these new males into the resident group of liontailed macaques at Fota is still ongoing. Studies have shown that the successful integration of unfamiliar individuals is a long, slow process. A study on the integration of two captive chimpanzee groups found that one year after the introduction, the two groups remained distinct from one another, supporting the idea that the development of positive social behaviour between unfamiliar individuals takes time (Schel et al. 2013). Cooper et al. (2001) found similar results with tufted capuchins, where affiliative relationships with the resident female took many weeks to establish. Bernstein (1969) reported differences between species of macaque, with pig-tailed macaque groups remaining unstable for weeks after group formation, much longer than rhesus macaques.

Similar to the study by Hoff et al. (1996), the long-term goal of the introduction at Fota was to encourage breeding and greater genetic diversity within the group. In 2018, one of the males (Mauzer) successfully bred with one of the females, therefore this introduction could be considered successful. There have been other behavioural issues, however; unlike the other male (Joe), Mauzer had not successfully integrated with the entire group. Mauzer had to be mixed with a small number of females either in the indoor house, or in the outdoor area of the enclosure. This was due to aggression that occurred when Mauzer was placed with the entire group that resulted in him escaping the enclosure. At the time of writing, however, Mauzer has been mixing peacefully with the entire lion-tailed macaque group, so the long-term success of this introduction looks promising.

Acknowledgements

The authors would like to thank all the staff at Fota Wildlife Park for help and advice in facilitating this research. The authors would also like to thank the Irish Research Council Enterprise Partnership Scheme and Fota Wildlife Park for funding this research.

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