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Pair Housing of Vervets/African Green Monkeys for Biomedical Research

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Abstract

Vervets, also known as African green monkeys, are a nonhuman primate species widely used in biomedical research. However, there are currently few references available describing techniques and rates of success for pair-housing this species. We present data from four cohorts of vervets from three different facilities: (i) the Wake Forest Vervet Research Colony (VRC; $n = 72$ female pairs, $n = 52$ male pairs), (ii) the University of Louisiana at Lafayette—New Iberia Research Center (UL-NIRC; $n = 57$ female pairs, $n = 54$ male pairs), (iii) the Tulane National Primate Research Center (TNPRC; $n = 18$ male pairs), and (iv) a cohort of imported males ($n = 18$ pairs) at Wake Forest. Compatibility was measured at 14, 30, and 60 days following introduction. Success rates for pair-housing at 14 days ranged from 96% to 98% for females and 96% to 100% for males at the VRC and UL-NIRC but were lower in the smaller imported male cohorts (TNPRC: 50%; WF: 28%). Among the UL-NIRC cohort and VRC male cohort, most of the pair separations after 14 days were due to reasons unrelated to social incompatibility. In contrast, a large proportion of TNPRC and imported male pairs successful at 14 days required separation within 60 days due to incompatibility. Multiple logistic regressions were performed using cohort, mean age of pair and weight difference between pair-mates as potential predictors of compatibility at 14 days. All three predicted the 14-day outcome in males but not females. A separate analysis in the VRC cohort found no evidence that prior familiarity in a group setting influenced outcomes. Variations in success rates across cohorts may have been influenced by introduction methodology. Behavioral

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differences between vervets and macaques, coupled with our findings, lead us to theorize that the gradual introduction techniques commonly implemented to pair house macaques may not be beneficial or suitable for this species.

Keywords

vervet monkeys; pair-housing; behavioral management

INTRODUCTION

Vervets (*Chlorocebus aethiops* sp.), also known as African green monkeys, are a nonhuman primate species widely used in biomedical research. However, there are few references available providing guidance for the best techniques for pair-housing this species. Given the current mandate of regulatory agencies regarding the fact that social housing should be the default housing arrangement for all nonhuman primate species, the goal of this paper is to begin to fill in the gap and provide much needed data on success rates for pair-housing this valuable nonhuman primate species.

Regarding nomenclature, many primatologists use the term “vervet” when referencing the genus *Chlorocebus*. In contrast, immunologists generally consider vervets to include only members of the subspecies (or species) *pygerythrus*, using “African green monkey” when referencing the genus as a whole. In this paper, we use the term “vervet” to refer to both the entire genus *Chlorocebus* as well as to the West African/Caribbean varieties (*C. sabaues* or *C. aethiops sabaues*)—a usage consistent with recent sequencing data [Jasinska et al., 2013].

According to Carlsson et al. [2004], *Chlorocebus aethiops* is one of the most frequently used nonhuman primate species in biomedical research. Although many of the studies cited by Carlsson et al. [2004] involved in vitro studies of commercially available cell cultures, a recent analysis of PubMed citations showed that the number of vervet citations was close to that of rhesus macaques and greater than any other nonhuman primate species [Jasinska et al., 2013]. The growth in the use of vervets for biomedical research may stem from the fact that they serve as an alternative to Indian-origin rhesus macaque. Vervets are relatively abundant, are not carriers of Herpes B virus, are easy to handle, and are less expensive than macaques [Baulu et al., 2002; Jasinska et al., 2013]. Vervets have been used in a wide variety of research areas including brain imaging [Fears et al., 2009; Woods et al., 2011], obesity and diabetes [Cann et al., 2010; Kavanagh et al., 2007], Alzheimer’s disease [Kalinin et al., 2013; Lemere et al., 2004], pharmacology/cognition [Groman et al., 2013; James et al., 2007; Melega et al., 2000, 2008], immunology [Pandrea et al., 2012; Zahn et al., 2008], and genetics [Freimer et al., 2007; Jasinska et al., 2012, 2013; Voruganti et al., 2013].

Regulations that require facilities to address the social needs of nonhuman primates have long been in place. The USDA Animal Welfare Act regulation 9 CFR 3.81 (a) states that plans for environmental enhancement “must address the social needs of nonhuman primates of species known to exist in social groups in nature” [USDA, 1991, p. 100]. Despite regulations and consensus that social housing should be the default housing condition for

nonhuman primates, a 2003 survey of housing conditions for different nonhuman primate species [Baker et al., 2007] showed that none of the 179 vervets from the six different facilities surveyed were housed socially.

More recently, the Eighth Edition of the Guide for the Care and Use of Laboratory Animals [NRC, 2011] emphasized the importance of social housing. Like the Animal Welfare Act, the Guide noted that “Appropriate social interactions among members of the same species (conspecifics) are essential to normal development and well-being.” The importance of social housing is frequently stated throughout the Guide, and the emphasis on social housing for all social species is evident. For primates specifically, the Guide states that “social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility” [NRC, 2011, p. 64]. The Eighth Edition of the Guide may be in part responsible for changes in the housing of vervets. For instance, among the three facilities participating in the current study and currently housing vervets, 50–97% of animals are socially housed [Personal Communications: Baker; Breaux; Jorgensen].

Both the Animal Welfare Act and the Guide recommend that behavioral management plans should be tailored to each species [see also Williams et al., 2015; Worlein et al., 2015]. The Guide states that “group composition is critical and numerous species-specific factors such as age, behavioral repertoire, sex, natural social organization, breeding requirements, and health status should be taken into consideration when forming a group” [NRC, 2011, p. 59]. In addition, the Guide emphasizes that “an understanding of species-typical natural social behavior (e.g., natural social composition, population density, ability to disperse, familiarity, and social ranking) is key to successful social housing” [NRC, 2011, p. 64]. However, despite the widespread and growing use of vervets for biomedical research and increases in the use of social housing, there are very few resources available describing the social management of this species, including methods for pair-housing. This may be due to the fact that vervets are relatively under-represented in the national primate research centers, where most of the expertise in nonhuman primate behavior and management tends to reside. Therefore, smaller labs, often without dedicated behavioral management teams, may be the primary users of these animals. Many facilities employ introduction methodology developed for the social management of macaques [Coleman et al., 2012; DiVincenti and Wyatt, 2011]. However, the effectiveness of managing vervet introductions using methodology developed for macaque species has not been evaluated.

The purpose of this paper is to provide a retrospective analysis of isosexual pair introduction outcomes from four different vervet colonies at three different biomedical facilities. Many of the pair-housing methods described herein have been derived without the use of rigorous scientific comparisons with other possible methods. Methods were based upon varying levels of expertise at the different facilities, in some cases based upon experience working with large multi-male, multi-female, matrilineal social groups housed in a captive environment.

METHODS

In order to ascertain the success rates for pair-housing vervets in a research setting, we examined male and female isosexual pairing attempts from four different cohorts of animals from three different facilities: (i) the Wake Forest Vervet Research Colony, which has two discrete cohorts, one with male and female subjects drawn from their longstanding group housed colony (abbreviated herein as VRC), and a cohort of males imported in 2009 from St. Kitts Biomedical Research Foundation in Basseterre, St. Kitts, West Indies, (ii) the University of Louisiana at Lafayette—New Iberia Research Center (NIRC), and (iii) the Tulane National Primate Research Center (TNPRC). See Table I.

Across all colonies, the success of pair introductions was defined as a pair still being housed together for at least 14 days after being given full-contact. Decisions to separate pairs due to social incompatibility were based on incidents of aggression and/or wounding and typically involved consultation between veterinary, enrichment/management, research and animal care staff. In addition, the housing status of initially successful pairs was measured at 30 and 60 days after introduction. In a biomedical or breeding context, pairs may be separated for a number of reasons unrelated to the social dynamics with a pair, such as transfer to different studies and/or housing areas, preparation for shipment to other facilities, or integration into breeding groups. When assessing continued compatibility, it is important to exclude pairs separated for what we term “practical reasons.” Therefore, the separation of pairs after 30 or 60 days was categorized as either due to social incompatibility or for “practical reasons” unrelated to incompatibility.

The research described in this manuscript adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates and protocols were approved by the appropriate Institutional Animal Care and Use Committees from each institution.

Cohort 1-Wake Forest Vervet Research Colony (VRC)

The first cohort consisted of known-age, colony-born animals from the Vervet Research Colony (VRC). The VRC is a captive population of Caribbean-origin vervets (*Chlorocebus aethiops sabaeus*) housed at the Wake Forest Primate Center (WFPC) at the Wake Forest School of Medicine. Currently, the VRC consists of two main components: matrilineal breeding groups ($n=271$ total) and a subset of animals housed in small groups, pairs and individually ($n=59$; 16 males in small groups, 32 pair-housed females, 11 individually housed females).

Cohort 1 consisted of 87 females aged 3.2–25.3 years old at first pairing (mean age = $10.8 \pm$ SD 5.5 years) and 100 males aged 2.8–10.6 years old at first pairing (mean age = $4.6 \pm$ SD 1.7 years). These animals were used to form 72 female–female pairs and 52 male–male pairs. Pairing took place between 2009 and 2014. Some animals were used in multiple pairs. In addition, 23 (32%) of the female pairs and 41 (79%) of the male pairs had prior familiarity with each other, having been housed together in the same social group prior to pair introductions.

Pair-housed animals in the VRC were typically maintained in four-cage racks. Cage sizes ranged in floor area from 0.4 m² to 0.6 m² per animal with built-in or re-fabricated cage dividers that allow visual-only contact, protected contact provided by mesh or perforated panels, or full contact between adjacent cages. Pair introductions occurred in rooms containing 2–24 animals per room.

The typical methodology for pair-housing involved the transfer of a fairly large cohort of same-sex animals from the matrilineal breeding groups to indoor pair-housing. For example, one of the first sets of pair-housed animals involved 38 females that were all removed from the social groups at the same time. Whenever possible, animals were moved into new rooms without any resident animals. There were no fixed criteria used for selecting pair-mates, and many pair selections were random. Animals were given full-contact on the day of transfer, without any protected contact. Barring the observation of aggression or distress, pairs were granted continuous contact from the day of introduction. Animals were observed daily by animal care, veterinary, and/or research staff as part of standard monitoring practices.

Cohort 2—UL Lafayette—New Iberia Research Center (UL-NIRC)

Cohort 2 consisted of 105 females aged 1.1–24.3 years old at first pairing (mean age = $4.1 \pm \text{SD } 2.7$ years) and 91 males aged 1.0–6.9 years old at first pairing (mean age = $2.8 \pm \text{SD } 1.4$ years) from a captive breeding colony maintained at UL Lafayette-NIRC. These animals were used to form 57 female–female pairs and 54 male–male pairs. Pairing took place between 2010 and 2014. Some animals were used in more than one pair. Animals were previously housed in mixed-sex social groups of two to seven individuals and were transferred indoors for same-sex pair-housing. It was not possible to determine how many pairs had been previously housed in the same social groups prior to pairing.

During introductions, animals were housed in one- or two-tiered wall mounted cages with a height of 0.8 m and either 0.4 m² or 0.6 m² of floor space. Each pair of cages had a removable solid panel and a removable grooming bar panel [Crockett et al., 1997] to allow for full contact and protected contact, respectively. Introductions were conducted in rooms containing 4–30 individuals.

For each pair attempt, the socialization process began with brief protected contact under supervision via grooming bar panel before proceeding to full contact under supervision (i.e., paired only when subject to direct observation), then to full contact without supervision. Progression from one stage of introduction to the next was determined on a case-by-case basis depending upon behavior observed. Duration of introductions ranged from one session (55% of pair attempts) to four sessions across several days. Pairs with multiple introduction sessions that did not display aggressive behavior under supervised protected contact were left with grooming bar access between sessions until progressing to full contact without supervision. Observation sessions ranged from 20 to 60 min with approximate total observation time of 1.5 hr per pair attempt. Exceptions were pairs displaying aggressive behavior which required immediate separation.

Cohort 3 — Tulane National Primate Research Center (TNPRC)

Cohort 3 consisted of 20 males aged 6.0–7.5 years old at first pairing (mean age = $6.6 \pm \text{SD } 0.5$ years).

All animals were transferred from other captive facilities and had been housed in single caging at the Tulane National Primate Research Center for at least 4 months before introduction. These animals were used to form 18 male–male pairs. Pairing took place between 2004 and 2012. Some animals were used in multiple pairs. Although animals were transferred from other captive facilities, to the best of our knowledge no pairs had prior familiarity with one another.

Individuals were housed in standard stainless steel racks containing four cages each. All cages had a height of 0.8 m and floor space of 0.4 m^2 per animal. Each cage had one side wall that could accommodate perforated panels permitting protected contact via holes in the panels, or could be removed to provide full contact. Subjects were introduced while housed in rooms containing between 4 and 21 individuals.

Pair introductions took a step-wise approach that is commonly implemented with macaques. All introductions began with a protected contact phase implemented with the use of perforated panels with twelve $5 \times 8 \text{ cm}$ holes. The protected contact phase generally lasted between 2 and 14 days (mean = 8.5 days), depending on whether aggression, distress, or grooming were observed. Once the introduction process began, pairs that did not display serious or escalating aggression or distress were left in contact from the first day of the introduction as opposed to having contact for a short period of time each day. On the first day of introduction, new pairs were directly monitored for approximately 1 hr followed by several observations of shorter durations later that day. Once the protected contact panel was removed, the same schedule of observation was implemented, with multiple daily checks until 2 weeks had passed.

One additional pairing attempt was unsuccessful prior to reaching limited full contact, and this pair was excluded from our analysis data set. Full contact pairs that required separation due to aggression were generally returned to (and in most cases successfully maintained in) protected contact in order to provide opportunity for interaction. In the present study, introductions with that outcome were coded as unsuccessful.

Cohort 4—Wake Forest Imported Males (WF-Imported)

Cohort 4 consisted of 25 male vervets that were imported to Wake Forest from the St. Kitts Biomedical Research Foundation (SKBRF) in 2009. Prior to arrival at Wake Forest, it is assumed that all animals were unfamiliar as they had a range of acquisition dates to the SKBRF. At the time of arrival, the males ranged in age from 2.5 to 9.0 years old at first pairing (mean age = $5.5 \pm \text{SD } 1.6$ years; all ages estimated). These males were used to form 18 pairs. The animals underwent an initial quarantine phase for approximately 5 weeks, before shifting locations and finishing out the final quarantine phase 8 weeks later. Socialization attempts were initiated shortly after the start of the final phase of quarantine. During quarantine, all animals were housed in single quadrants of four-cage racks that provided at least 0.6 m^2 per cage.

Once released from quarantine, the animals were used on a protocol that involved a diet study which required daily separation to account for how much each individual animal ate. Any successful pairs were also temporarily separated for study procedures (generally not longer than 24–48 hr), including the collection of urine, feces, and/or sample collections requiring sedation events. Cage sizes and types were identical to the VRC cohort. Pair introductions were performed in a room that contained only the 25 animals in this cohort.

Pair introductions took a similar step-wise approach to that done with macaques. The animals to be paired were first given visual access only for 1–14 days. If no aggressive behaviors were noted, animals were given full contact for a limited period of time (generally from 10 AM to 2:30 PM to coincide with separations for feeding) with no overnight or weekend contact. This limited full contact was provided for 1–8 weeks depending on the situation. If neither animal showed signs of injury or distress during this phase, they were granted full contact with overnight and weekend access.

Three additional pairing attempts were unsuccessful prior to reaching limited full contact, and these attempts were excluded from our analysis data set. We did include the 14 pairs that were separated during limited full contact. These pairs were coded as 0 days duration, although they never had full contact with access overnight and on weekends.

Statistics

Associations between vervet pair characteristics and success of pair housing at 14 days were tested using Fisher's exact test. These associations were further evaluated using multiple logistic regression models, to account for the characteristics simultaneously as independent covariates. Association results of the modeling were expressed as adjusted odds ratios (95% confidence intervals) for each independent variable: (i) sex, (ii) cohort, (iii) the average age of the pair, and (iv) absolute difference in body weight between the pair-mates. Age, body weight, and sex were selected as independent variables based on previous work in macaques [e.g., Carlson 2008; DiVincenti & Wyatt, 2011]. These variables were also similar to the covariates used by Capitanio et al. [2015]. Age differences between pair-mates, and average weights, were not used in the modeling, although they were highly correlated with the other covariates.

First, an overall model was explored using all pairs. No interaction effects were tested; however, although female pairs were derived from only a subset of cohorts, multiple logistic regression models were then applied to male and female pairs separately. This also allowed for separate odds ratio estimates in male and female pairs. Some animals appeared in multiple pairs, which is a possible violation of the assumption of independence for the regression analyses. Therefore, we performed separate analyses excluding all pairs representing a repeated appearance of an animal. Unless otherwise noted, analyses of the subset of pairs were similar to results found in all pairs, and only results from the larger data set are reported. Differences at the 30 and 60 day time periods were examined using descriptive statistics. All statistical analyses were performed using either SAS version 9.4 (SAS Institute Inc., Cary, NC), Stata version 14 (StataCorp, College Station, TX), or R version 3.1.1 [R Core Team, 2013]. *P* values <0.05 were considered statistically significant.

RESULTS

Across all 271 pairs, 90% of the introductions were successful, that is, animals were still housed together after 14 days. Female pairs were more successful than male pairs overall (94% of female pairs, 83% of male pairs, $P < 0.001$); however, there was considerable variation in pair-housing success across male cohorts (Table I: range 28–100%, $P < 0.001$). In the two cohorts that included both male and female pairs, pair housing success did not differ between males and females for the VRC cohort ($P = 0.26$) or the UL-NIRC cohort ($P = 0.61$).

Table II shows the results of the multiple logistic regression analyses. For the overall data set that included both male and female pairs, the odds of success were greater in pairs with younger mean age, adjusting for sex, weight difference between pair-mates, and cohort ($P < 0.0001$). Weight difference was not statistically significant, adjusting for sex, mean age, and cohort ($P = 0.055$). Due to the different composition of cohorts with respect to male and female pairs, effects of cohort were evaluated within the sex-specific analyses.

Among facilities housing males, there was 100% success at 14 days for the VRC cohort, which was not significantly different from the rate among males at the UL-NIRC ($P = 0.50$). However, this success rate was significantly different from the TNPRC ($P < 0.0001$) and the WF imported ($P < 0.0001$) cohorts. Using the three cohorts with variability in success, the multiple logistic regression showed overall differences among these remaining cohorts ($P = 0.0057$; Table IIB), with increased odds of success in both the UL-NIRC and TNPRC male groups compared with the WF imported males, adjusting for mean age and weight differences. Similar to the analyses in males and females combined, in males, the odds of success decreased with increased mean age. Adjusted for cohort and weight differences, the odds of success were approximately 0.5 times lower on average for pairs with an older mean age by 1 year. For weight differences, the odds of success were significantly increased by 3.6 times for each kilogram difference, adjusting for cohort and mean age.

For females, data were available for the VRC and UL-NIRC cohorts only. Unlike the males, age and weight difference were not significantly associated with pair-housing success in females in the multiple logistic regression modeling (Table IIC). The cohort effect also was not statistically significant in females ($P = 0.66$, Table IIC), which is similar to the comparable test result across these two cohorts in males ($P = 0.50$, Fisher's exact test).

In order to describe how long successful pairings remained compatible in vervets and to distinguish between separations due to social incompatibility and those due to practical reasons, we examined the social status of pairs at 30 and 60 days following initial social introduction. Figure 1 depicts not only initial success but also the proportion of pairs (i) still successfully paired, (ii) separated due to social incompatibility, or (iii) separated due to reasons unrelated to incompatibility. These results are described here by cohort.

Cohort 1—Wake Forest Vervet Research Colony

Females—96% of the VRC female pairs were initially successful. By 30 days, 89% of these pairs remained intact. Excluding pairs separated for practical purposes, 94% remained

compatible; other pairs had been separated due to fighting or distress between 14 days and 30 days postintroduction. By 60 days, 82% of originally successful pairs remained intact and 94% of pairs not separated for practical reasons between 14 and 30 days remained intact (see Fig. 1).

Males—At 30 days, 98% of originally successful pairs remained intact, owing only to separation for practical reasons. By 60 days, 75% of pairs remained intact, again only due to separation for practical reasons.

Effects of prior familiarity

Females—Success rates were compared between pairs in the VRC cohort that had prior familiarity (previously housed in the same social group) and those that were unfamiliar with each other. For females, 1 of 23 (96%) of familiar pairs and 2 of 40 (96%) of unfamiliar pairs were successful after 14 days. For familiar pairs, 91% were intact after 30 days and 87% after 60 days. For unfamiliar pairs, 88% were intact after 30 days and 80% after 60 days. Two of the three familiar female pairs were separated due to social incompatibility, whereas 9 of the 10 unfamiliar females pairs were separated due to social incompatibility.

Males—For males, 100% of pairs were successful after 14 days. For familiar pairs, 100% of pairs remained intact after 30 days and 81% after 60 days. For unfamiliar pairs, 91% were intact after 30 days and 55% after 60 days. Unlike females, none of the unsuccessful male pairs were separated due to social incompatibility.

Cohort 2—UL Lafayette—New Iberia Research Center

Females—As shown in Figure 1, 98% of the UL-NIRC female pairs were successful after 14 days and all pairs remained intact at 30 days. No pairs required separation for incompatibility. By 60 days, 77% of pairs remained intact, due only to separations for practical reasons.

Males—96% of the UL-NIRC male pairs were successful after 14 days. As with females, all pairs remained intact at 30 days. By 60 days, 54% of pairs remained intact, again with separation only due to practical reasons.

Cohort 3—Tulane National Primate Research Center

The success rate for small cohort of males from the TNPRC was 50% after 14 days (see Fig. 1). Practical reasons did not interfere with pairings in this cohort. Incompatibility between days 14 and 30 reduced the percentage of intact pairs to 6%. The remaining pair was still intact at 60 days.

Cohort 4—Wake Forest Imported Males

Only 28% of the pairs in the imported male cohort were successful at 14 days. Practical reasons did not interfere with pairings in this cohort. As shown in Figure 1, none of these animals required separation by 30 days. By 60 days, only 17% of pairs remained compatible.

DISCUSSION

To our knowledge, this paper describes the first survey of pair-housing success rates in vervets. “Success” was defined as pairs housed together 14 days after full-contact introductions. Success rates were generally high by this metric in the larger VRC and UL-NIRC cohorts (96–98% for females, 96–100% for males) but were lower in the smaller TNPRC and WF imported male cohorts (28–50%). Analysis of prior familiarity, only possible in the VRC cohort, indicated that animals that had been previously housed together were no more likely to be successfully paired than animals that were previously unfamiliar with each other.

The results of multiple logistic regression analyses indicated that age was a significant factor in predicting pair-housing success. Comparisons of male and female pairs in cohorts with both types of pairs showed no significant differences in success by sex. In logistic regression analyses within each sex separately, the results showed that cohort, age, and weight difference were all significant predictors of pair-housing success in males but none of those predictors were significant in females. This suggests that the factors influencing pair-housing success in males may be quite different than for females. This is consistent with observations of dominance acquisition in cercopithecines in which factors such as size and strength are important for males but less so for females [Walters & Seyfarth, 1987]. One caveat for this in our analysis, however, is that female pairs were not represented in the two cohorts with lower success rates in males.

Our data also showed that at some facilities, research, breeding, or sale of animals may disrupt the continued pair housing of vervets. In most cohorts, success at 14 days appears to predict long-term success, the exception being the two cohorts with very low success rates that also used step-wise introductions. By 60 days, later compatibility plays a very small to nonexistent role in the separation of pairs, with the exception, again, of the cohorts with low success rates at 14 days. These findings may suggest that the step-wise method of introducing vervets may have long-lasting effects on relationship formation and stabilization.

Possible Factors Contributing to Varying Pairing Outcomes Across Facilities

The lowest success rates were observed in the cohorts of imported males at the TNPRC and WF. Although both of these cohorts included relatively few animals which may limit the implications of these findings, there were several ways in which cohorts at these facilities were different from the others. Animals at these two facilities were imported and experienced varying durations of single housing. In some cases, their status as wild- versus captive-born was in question. In addition, they had undergone housing in multiple facilities as well as transportation stress. Within these populations, there was considerable variety in the length of time at the previous vendor prior to shipment. The length of time spent at the vendor prior to shipment varied from 15 days to nearly 6 years. If the animals had been wild caught prior to arrival at the vendor facility, it could be that some of the animals were still acclimating to captivity, whereas others were more experienced.

In addition, the cohorts varied with respect to whether new pair-mates had prior familiarity in a group setting. However, our results from one cohort in which some pairs had prior familiarity suggest that it may not in fact influence outcome, although a more controlled comparison of the pairing of familiar and unfamiliar animals would be necessary to test this suggestion. An examination of prior pair-housing successes or failures would also be useful.

These cohorts also differed from the others in the introduction methods used to form pairs. At the TNPRC and WF, a step-wise introduction plan was employed. As Carlson [2008], Coleman et al. [2012], DiVincenti and Wyatt [2011], and Truelove et al. [2015] have described, typical socialization attempts with macaques proceed in a step-wise fashion allowing “pair familiarization” during which both members of the pair are allowed to establish a dominant–subordinate relationship with little to no physical or tactile contact. The animals are allowed to see each other without contact, and the amount of tactile access is steadily increased until the animals are in full contact. The period of protected contact can last for a few hours to a few days or even weeks.

The Role of Species Differences

When compared with reported success rates in macaques, the range of success rates found in the current study (28–100%) exceed the variability in adult isosexual pairs seen in any one species of macaque. Published success rates for rhesus macaques vary from 57% to 100% (reviewed in Carlson [2008] and DiVincenti & Wyatt [2011]; also see Baker et al. [2012] and Capitanio et al. [2015]). In addition, some of the success rates reported in this paper are lower than any reported success rates for *Macaca mulatta* and *Macaca fascicularis* [Capitanio et al., 2015; Crockett et al., 1994]. This variability suggests cohort composition and characteristics (which vary between studies for macaque species as well) may be less responsible than the differences in methodology used. If true, the findings of the current study may be very valuable for guiding the methodology of pair formation in vervets. Those trying to pair-house vervets should be wary of what we here term a “macaque-centric” approach to pairing, focused on gradual introduction of animals.

Why might macaque-centric behavioral management techniques not always be appropriate for vervets? There is a wealth of data that exists on techniques and factors to consider when pair-housing macaques, and we assume that most personnel new to social housing vervets are probably more familiar with the behavior patterns of macaques than vervets. Although knowledge of macaque behavior may be useful for some aspects of vervet behavioral management, we are concerned that over-generalizing may not be ideal. Vervets and macaques have several potentially significant differences in behavior. First, the behavioral repertoires of the two species are not identical. One prime example of behavioral differences pertains to the often confusing “hugging” behavior seen in vervets. In the early years of the VRC, Fairbanks et al. [1978] called this behavior “handling” and described it as “touching another individual with the hands or embracing with arms including placing hands on head or shoulders, embrace, and straddle, excluding touching the genitals.” Fairbanks & McGuire [1986] later called this behavior “manipulate” and described it as “hands-on-head, hands-on-shoulders, and embrace-from-in-front” citing similar descriptions of this behavior by Struhsaker [1967] and Rowell [1971]. This is a relatively stylized set of behaviors that may

be followed by grooming or will sometimes lead to aggression [Struhsaker, 1967]. This behavior may be misunderstood as simply an affiliative gesture and could lead to introduction decisions that are not guided by accurate interpretation of the unfolding social interactions. In addition, there may be broad differences between the taxa in the expression of affiliation and aggression, in the strategies used to form bonds and dominance relationships, or response to strangers. For example, vervets tend to have more subtle dominance displays and a less rigid dominance hierarchy [Fairbanks & McGuire 1986; Rowell 1971].

RECOMMENDATIONS AND CONCLUSIONS

It is beyond the scope of this paper to explore what underlying species differences may result in varying responses to the macaque-centric introduction technique. However, we speculate that if vervets form social bonds in fundamentally different ways from macaques, protected contact will not serve a useful function in this species. For example, one function of a period of protected contact during pair formation is to allow conflict and determination of rank relationships to occur in a setting in which individuals can withdraw, but also permits some bond development prior to allowing animals to share the same physical space. In our experience, vervets rarely show overt aggression on the day of introduction, and it may be days or weeks before any aggression begins. Therefore, a relatively brief period of protected contact may not allow managers to terminate ultimately incompatible pairs prior to full access, eliminating a main benefit of this technique. We theorize that unlike macaques, if physical interactions are critical for establishing dominant–subordinate relationships in vervets, the use of a familiarization period with vervets may simply increase anxiety or frustration and not increase the safety of introductions. In other words, protected contact may only constrain normal social interchange in vervets.

Because data presented in this paper did not involve controlled comparisons of different techniques, future research is needed to determine the role of age and weight toward guiding the selection of potential partners and predicting outcomes. Most importantly, perhaps, research is needed to determine whether or not immediate full-contact introductions of new pairs are in fact superior to “macaque-centric” techniques. In conclusion, the data presented in this paper, along with other papers in this special issue, will generate interest in exploring ways in which pairing strategies should be tailored to species. The suggestions made herein may help guide behavioral scientists, other researchers, veterinary staff, and behavioral management staff who are using this unique species.

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Fig. 1.

Percent successful pair-housing by cohort at 14, 30, and 60 days post-introduction.

“Successful pair,” pairs housed together; “separated for incompatibility,” pairs separated due to social incompatibility; “separated for other reasons,” pairs separated for reasons other than social incompatibility. See text for details

TABLE I

Description of Pair-Housing Cohorts

Cohort	Facility	Sex	Number of pairs	Number of animals	Age (years)	Weight (kg)	Period of protected contact	Pairs with prior familiarity	Successful pairs at 14 days	95% confidence interval
1	WF VRC	F	72	87	10.8 ± 5.5	5.34 ± 1.04	No	32%	69 (96%)	(88.5, 98.6)
1	WF VRC	M	52	100	4.6 ± 1.7	6.32 ± 1.36	No	79%	52 (100%)	(93.1, 100)
2	UL-NIRC	F	57	105	4.1 ± 2.7	3.30 ± 0.92	Yes	Unknown	56 (98%)	(90.7, 99.7)
2	UL-NIRC	M	54	91	2.8 ± 1.4	3.28 ± 1.56	Yes	Unknown	52 (96%)	(87.5, 99.0)
3	TNPRC	M	18	20	6.6 ± 0.5	7.16 ± 0.64	Yes	Assumed 0%	9 (50%)	(29.0, 71.0)
4	WF imported	M	18	25	5.5 ± 1.6	6.51 ± 0.79	Yes	Assumed 0%	5 (28%)	(11.8, 48.8)

Results reported as mean ± standard deviation. WF-VRC, Wake Forest Vervet Research Colony; UL-NIRC, University of Louisiana New Iberia Research Center; TNPRC, Tulane National Primate Research Center.

TABLE II

Multiple Logistic Regression Results Predicting Pair Housing Success at 14 Days

A. All pairs (<i>n</i>=271), 4 cohorts			
Parameter	Odds ratio	Confidence interval	P-value
Sex (female vs. male)	20.4	(3.70, 112)	0.0005
Cohort/method (VRC vs. other cohorts)	36.5	(4.65, 287)	0.0006
Mean age (1-year increment)	0.670	(0.570, 0.787)	<0.0001
Weight difference (1-kg increment)	1.77	(0.989, 3.18)	0.055

B. Males, <i>n</i>=90 pairs, 3 cohorts (VRC cohort excluded)			
Parameter	Odds ratio	Confidence interval	P-value
Cohort (UL-NIRC vs. TNPRC)	1.90	(0.133, 27.0)	0.0057 *
Cohort (UL-NIRC vs. WF imported)	26.4	(2.28, 305)	
Cohort (TNPRC vs. WF imported)	13.8	(1.85, 103)	
Mean age (1-year increment)	0.465	(0.238, 0.907)	0.025
Weight difference (1-kg increment)	3.61	(1.04, 12.6)	0.044

C. Females, <i>n</i> = 129 pairs, 2 cohorts **			
Parameter	Odds ratio	Confidence interval	P-value
Cohort (VRC vs. UL-NIRC)	0.519	(0.027, 9.94)	0.66
Mean age (1-year increment)	0.957	(0.748, 1.22)	0.73
Weight difference (1-kg increment)	1.33	(0.332, 5.30)	0.69

VRC cohort excluded from Male dataset due to limited variability in success at 14 days.

* Overall test for a difference among all three cohorts (UL-NIRC, TNPRC, WF imported).

** No significant explanatory variables were found among mean age, age difference, mean weight, and weight difference. All adjusted for cohort (VRC vs.UL-NIRC).