

Comparison of Two Introduction Methods for African Elephants (*Loxodonta africana*)

Kyle D. Burks,^{1,2*} Jill D. Mellen,^{1,2} Gary W. Miller,¹ John Lehnhardt,¹ Alexander Weiss,^{3,4} Aurelio J. Figueredo,³ and Terry L. Maple^{5,6}

¹Disney's Animal Kingdom, Lake Buena Vista, Florida

²Department of Biology, University of Central Florida, Orlando, Florida

³Department of Psychology, University of Arizona, Tucson, Arizona

⁴National Institute on Aging, National Institutes of Health, Department of Health and Human Services, Baltimore, Maryland

⁵School of Psychology, Georgia Institute of Technology, Atlanta, Georgia

⁶TECHLab, Zoo Atlanta, Atlanta, Georgia

Managers must consider an animal's potential for aggression when they decide to change or form a captive social group formation. In this study we compared two introduction methods (termed "sequential" and "nonsequential" introductions) in African elephants to assess their effectiveness in managing aggression and minimizing stress. Both introduction methods included four phases: baseline, visual contact, limited tactile contact, and physical introduction. In the sequential introduction, these steps were followed sequentially, and empirical data were considered during decision-making. In the nonsequential introduction, these steps were not followed sequentially, and decision-making was based primarily on intuitive assessments by animal managers. Behavioral data and fecal corticoid concentrations were measured throughout both types of introduction. The behavior categories measured included active aggression, passive aggression, submissive behavior, undesirable/stress-related behavior, and affiliative behavior. While the role of affiliative behavior was surprising, general behavior patterns were characterized by increases in behavior as animals progressed to the next phase of introduction regardless of introduction type. These increases then attenuated over time during each phase. Overall, less behavior was observed during the sequential introduction, as predicted. The data suggest that the sequential introduction managed aggression more

*Correspondence to: Kyle Burks, Ph.D., Disney's Animal Kingdom, P.O. Box 10,000, Lake Buena Vista, FL 32830. E-mail: kyle.burks@disney.com

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effectively. Similar patterns were predicted for undesirable/stress-related behavior and fecal corticoid concentration. Undesirable/stress-related behavior was a poor predictor of observed behavior patterns. Although the patterns differed from those predicted, higher concentrations of fecal corticoids were measured during the nonsequential introduction and correlated significantly only with submissive behavior. While more investigation is warranted, the data indicate that the nonsequential introduction brought about an increased physiological response. Overall, the sequential introduction method appeared to manage aggression and stress better than the nonsequential technique. Every introduction is subject to factors that can influence success, such as staff experience, the design of the facility, and the animals' social histories. It is hoped that the rigorous sequential protocol will be a useful tool in the animal manager's "toolbox" for planning and implementing introductions. Applications of this introduction method are also discussed. *Zoo Biol* 23:109–126, 2004. © 2004 Wiley-Liss, Inc.

Key words: aggression; behavioral management; socialization; group formation

INTRODUCTION

The behavioral, genetic, and demographic management of captive species in zoos has led to a frequent need to change the composition of social groups. Historically, decisions regarding the introduction process have been made by well-informed and experienced individuals who rely primarily on past experience and their intuitive assessment of the current situation.

Most research focused on group formation in captive wild animals has been conducted on primate species, and two general approaches have been used. One approach is to immediately place the animals in the same physical space (for review see Bernstein [1991]). Groups formed in this manner have patterns of behavior dominated initially by aggression and responses to aggression. Other behaviors, such as affiliative behaviors, tend to be infrequent at the time of introduction, although their frequency increases over time as aggression decreases. Although some researchers have reported variations in this pattern [Mendoza and Barchas, 1983; Saltzman et al., 1991; Lyons et al., 1994], classic studies of group formation have indicated that the potential for aggression between unfamiliar individuals is high, and this must be considered in the planning and implementation of animal introductions.

As zoos began to shift toward more applied methods of managing introductions, Lindburg [1986] suggested that physical and social environments should be manipulated to meet the immediate goal of managing aggression and minimizing stress. This approach has led animal managers to provide animals with some period for social interaction prior to introduction into a shared physical space. Such introductions have met with varied success, primarily based on whether the protocols used were systematic and rigorously followed. Although managers have taken steps to allow social experience through visual, auditory, olfactory, and limited tactile contact, introductions have been documented (e.g., in gorillas [Bowen, 1981; Sunde and Sievert, 1995]) that did not apply systematic ordering of these steps. Aggression either persisted for long periods, resulting in lengthy introductions involving serious physical aggression [Sunde and Sievert,

1995], or resulted in incompatibility of some members in the group [Bowen, 1981]. While introductions can be successful without the use of rigorous protocols, the potential for aggression and stress warrants the development of more systematic approaches to assist in decision-making. Various introduction methods have involved the use of rigorous protocols and clearly defined behavioral criteria to assist in decision-making [Fritz and Fritz, 1979; Fritz, 1989; Winslow et al., 1992; McDonald, 1994; Alford et al., 1995; Bloomsmith et al., 1999; Seres et al., 2001]. In these instances, systematic and controlled protocols and an experienced staff were beneficial for long-term success. However, the decision-making criteria were based primarily on subjective assessment.

Empirical Model to Drive Decision-Making

Burks et al. [2001] expanded on a method described by Winslow et al. [1992] to gather empirical data during the socialization of an adult male gorilla that was a long-term social isolate. This method allows for more objective assessment and the use of empirical data during decision-making. Their introduction protocol included four discrete phases, which are often used in captive situations: 1) baseline, 2) visual contact (consisting of visual, olfactory, and auditory contact), 3) limited tactile contact, and 4) physical introduction. Behavioral data were collected in each phase of introduction for three behavioral categories: active aggression (which involved rapid body movement and/or contact with another individual), passive aggression (no rapid body movement or physical contact), and affiliative behavior. In general, increases in aggressive behavior were observed at each transition to the next phase of introduction, and aggression then declined toward baseline levels. Increases in aggression were more pronounced as introduction phases allowed for more physical contact. In general, affiliative behavior was not observed to vary systematically throughout the introduction process. Overall, the introduction method effectively managed aggression in a situation where abnormally high aggression was expected from a long-term social isolate. Burks et al. [2001] advocated further use of this rigorous protocol and suggested that data collection be used to augment decision-making in different contexts.

In this study we compared two introduction methods during the formation of a herd of 0.6 African elephants (*Loxodonta africana*): 1) the empirical methodology described by Burks et al. [2001], and 2) a nonsequential method. The “sequential” method systematically followed the introduction protocol of sequential introduction phases, and the other (“nonsequential”) varied the order of introduction events based on the intuitive assessments of animal managers. Application of the methodology of Burks et al. [2001] necessitated some modifications to the examined measures. In Burks et al.’s [2001] study of the socialization of a long-term isolate adult male gorilla with females, the male was presumed to be the primary aggressor. However, in situations where no clear aggressor can be identified a priori, the addition of submissive behavior to the data collection protocol would be beneficial. Additionally, measuring undesirable stress-related behaviors enables the effects of the introduction method on stress to be examined. As a complement to behavioral measures, the use of physiological measures can provide additional information concerning minimization of stress to individuals.

Glucocorticoid concentrations have been monitored in a number of species as an indicator of stress, and conditions of uncertainty, lack of control, and unpredictability of future events have been shown to be potent stressors [Levine et al., 1978]. Social group formation [Levine, 1993] and dominance interactions [Sapolsky, 1983] are known to elicit elevated glucocorticoid excretion. All of these potential stressors are relevant in animal introductions.

Social Organization of African Elephants

African elephants live in matriarchal family units composed of one or more related females and their offspring [Buss, 1961; Douglas-Hamilton and Douglas-Hamilton, 1975], which constitute the smallest organizational unit of a larger social structure [Douglas-Hamilton and Douglas-Hamilton, 1975; Moss and Poole, 1983]. Affiliation between groups is directly related to relatedness between groups [Moss and Poole, 1983]. Thouless [1996] found that unrelated social groups rarely approached within 2 km of each other.

Within each elephant social group, females use various forms of aggression (ranging from displacements to more overt physical aggression) to establish social rank and inhibit the reproductive efforts of subordinate females [Dublin, 1983]. In captivity, aggression plays a distinct role in social dynamics as well [Adams and Berg, 1980]. The majority of the North American African elephant population were wild-caught as infants, and thus most of the females have matured in the absence of the strong familial bonds observed in the wild. Thus, captive groupings are somewhat artificial, when compared to evidence from the wild that unrelated females do not interact frequently [Moss and Poole, 1983; Thouless, 1996] and that even under extreme circumstances of disruption in the natural social system, unrelated females will interact for only very short periods of time [Prins et al., 1994].

Given evidence from the wild that unrelated female elephants rarely interact, and data indicating that aggression is used to both establish and maintain social rank, one could predict that captive female African elephants will exhibit increased levels of aggression during group formation. Therefore, a modified version of the methodology described by Burks et al. [2001] appeared appropriate for use in investigating two approaches to introductions in this species. One method involved decision-making based primarily on intuitive assessments by animal managers, while the other method rigorously and systematically followed the introduction steps as outlined in the protocol.

The following hypotheses were tested during this study:

1. Affiliative behavior would not vary systematically during either introduction.
2. Within the phases of a nonsequential introduction, systematic decreases over time will not be observed in rates of active or passive aggression, undesirable/stress-related behavior, or fecal corticoid concentration. Alternatively, behavior rates and corticoid concentrations will decrease at a slower rate compared to those in a sequential introduction.
3. There would be more pronounced increases of undesirable/stress-related behaviors and fecal corticoid concentrations in a nonsequential introduction as compared to a sequential introduction.

TABLE 1. Subject information

Subject	Studbook Number	Time at previous institution	Time housed with conspecific	Immediate Social history	Age at transfer to dak
Thandi	195	Jan 1983–Jul 97 Point Defiance Zoo and Aquarium	1983–1997	Housed in a barn with tactile contact with 2 adult female Asian elephants (<i>Elaphus maximus</i>)	~16
Moyo	205	Same as Thandi	1983–1997	Same as Thandi	~15
Bala	189	1983–Oct 97 Phoenix Zoo	1983–1997	Housed with an adult female Asian elephant until 1996	~18
Fiki	190	Same as Bala	1983–1987	Same as Bala	~18
Petunia	127	1975–Feb 98 Knoxville Zoo	1975–1998	Tactile contact with an adult male African elephant	~25
Robin	92	1972–Feb 98 Knoxville Zoo	1975–1998	Same as Petunia	~22

MATERIALS AND METHODS

Subjects

The subjects of this study were 0.6 African elephants (*Loxodonta africana*). All of the females had been wild-caught and lived in pairs at their originating institution prior to their arrival at Disney's Animal Kingdom (DAK). Table 1 provides information on the study subjects and their social histories. The females arrived at DAK in pairs between July 1997 and February 1998.

Facilities and Housing

During the baseline phase of both introductions, the subjects were observed outdoors. Because Thandi and Moyo arrived at DAK several months before any other individuals, initial baseline data were collected after shipment while they occupied one or more of three outdoor yards at DAK. These yards measured 18.3×16.5 m, 18.3×20.1 m, and 27.4×21.9 m, respectively, and each contained a water source. During the baseline phase of the nonsequential introduction, Bala and Fiki were observed in an exhibit (~0.4 ha) that was furnished with large rocks and a water source. Baseline data during the sequential introduction for Thandi, Moyo, Bala, and Fiki were collected at DAK prior to the arrival of Robin and Petunia, in similar conditions to those described for Thandi and Moyo during the nonsequential introduction. Robin and Petunia were observed at the Knoxville Zoo in an approximately 0.2 ha exhibit furnished with large rocks and a water source during the baseline phase. After shipment, all elephants were housed in a large indoor holding facility with access to outdoor areas, and were housed individually overnight (see Fig. 1).

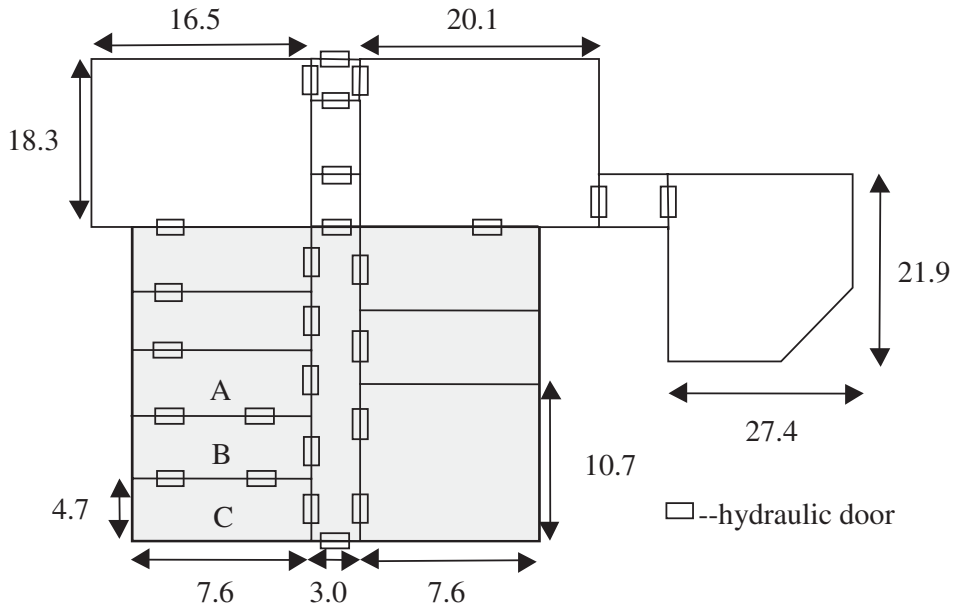


Fig. 1. Schematic diagram of Disney's Animal Kingdom's indoor (shaded) elephant stalls and outdoor holding yards. Stalls indicated by A, B, and C were used during indoor physical introduction periods. Not drawn to scale.

Experimental Design

Introduction phases

Using the methodology described by Burks et al. [2001], four phases of introduction were used throughout the study. After baseline data were collected, subsequent phases were initiated after unfamiliar individuals arrived at DAK. These phases included visual contact (consisting of visual, auditory, and olfactory contact with conspecifics across a hallway); limited tactile contact (individuals were placed in a "howdy" situation, i.e., they were allowed limited contact through ballards separating adjacent stalls or yards); and physical introduction (individuals were placed in the same physical space, either outdoors or in stalls A, B, or C (see Fig. 1)).

Nonsequential introduction

In the first introduction, Thandi and Moyo were introduced to Bala and Fiki. The phases of the introduction protocol were not followed in the order described by Burks et al. [2001]. Because of the long delay between the arrival of the first pair of elephants to DAK and the arrival of unfamiliar animals, it was deemed that the most appropriate baseline data for Thandi and Moyo were those collected after their arrival at DAK. After the baseline data were collected, animal managers chose the order of introduction phases on a daily basis based on their intuitive assessments of which phases should occur. The phases and the order chosen did not necessarily

TABLE 2. Summary of nonsequential elephant introduction events in chronological order

Introduction event	Subjects	Time frame
Baseline	Thandi, Moyo	9/17/97–10/3/97
	Bala, Fiki	9/2/97–9/11/97
Visual contact	All Four Subjects	10/7/97
Physical introduction	Fiki–Thandi	10/8/97–10/16/97
Tactile contact	Moyo–Bala	10/8/97–10/11/97
	Thandi–Bala	10/12/97–10/16/97
Physical introduction	Moyo–Bala	10/12/97–10/13/97
Tactile contact	Fiki–Moyo	10/16/97
Physical introduction	Thandi–Bala	10/16/97
	Moyo–Bala	10/17/97–10/22/97
Tactile contact	Thandi–Fiki	10/19/97–10/25/97
	Moyo–Fiki	10/25/97
	All four subjects	10/25/97
	Moyo–Fiki	10/26/97
	All four subjects	10/26/97
	Moyo–Fiki	10/27/97
	All four subjects	10/27/97–11/3/97
	All four subjects	11/4/97–11/11/97
Physical introduction	All four subjects	11/4/97–11/11/97

follow the sequential steps of the introduction protocol systematically. For a summary of the introduction phases and time frames, see Table 2.

Sequential introduction

The protocol for introductions described by Burks et al. [2001] was rigorously followed during the second introduction of the initial four females to Petunia and Robin. All introduction phases occurred in the order outlined by the protocol. Decisions to move forward in the introduction were based on daily examination of the behavior patterns observed, and intuitive assessments by animal managers. The primary objective criteria used were trends in aggression. In general, the staff examined the data daily, and moved forward in the introduction when systematic decreases in aggression were observed after each new introduction phase. For a summary of events during the second introduction, see Table 3.

Data Collection Procedures

Protocol for behavioral data collection

Behavioral data were collected using The Observer base package data collection software, version 3.0 (Noldus Information Technologies, Wageningen, The Netherlands) in conjunction with a hand-held Psion Workabout computer utilizing the Psion support package for The Observer. The Psion Workabout was configured as an event recorder to record the frequency of the behaviors described in the ethogram in Table 4.

Behavioral data were collected in five categories: active aggression, passive aggression, submissive behavior, undesirable/stress-related behavior, and affiliative behavior. Aggression was divided into two separate categories to distinguish between those behaviors that could lead directly to physical injury (e.g., active aggression)

TABLE 3. Summary of sequential elephant introduction events in chronological order

Introduction event	Subjects	Time frame
Baseline	Thandi, Moyo, Bala, Fiki	11/20/97–12/9/97
Visual contact	Petunia, Robin	1/26/98–2/1/98
Tactile contact	All Six Subjects	2/21/98–2/27/98
	Petunia–Thandi	3/1/98
	Petunia–Moyo	
	Petunia–Fiki	
	Petunia–Bala	
	Robin–Thandi	3/1/98
	Robin–Moyo	
	Robin–Fiki	
	Robin–Bala	
	Petunia–Thandi	3/2/98
	Petunia–Moyo	
	Petunia–Fiki	
	Petunia–Bala	
	Robin–Thandi	3/3/98
	Robin–Moyo	
	Robin–Fiki	
	Robin–Bala	
	Petunia, Robin–Thandi, Moyo, Bala, Fiki	3/4/98–3/7/98
Physical introduction	Petunia–Thandi	3/8/98
	Petunia–Moyo	
	Petunia–Fiki	
	Petunia–Bala	
	Robin–Thandi	3/9/98
	All six subjects	3/9/98–3/24/98

and those that did not involve active physical contact (e.g., passive aggression). When it was appropriate, initiators and recipients of behavior were recorded. Decisions regarding specific behaviors and their categorization were based on discussions between the first author and the elephant-care staff (keepers, zoo managers, and curator).

A focal-animal sampling technique [Altmann, 1974] was used, and observation periods were 60 min in length unless it was deemed necessary to intervene because of problematic aggression. A daily introduction plan was agreed upon by researchers and animal managers. According to this plan, observation periods were scheduled to balance data collection across time periods as much as possible. However, this was influenced by husbandry needs and the specific daily plans for introduction. Throughout the visual contact phase, with the exception of time periods when animals were shifted for husbandry purposes, such as cleaning, all subjects remained in visual contact with conspecifics. The length of the limited tactile contact sessions varied and was controlled by animal managers. After these sessions, individuals were returned to their home stalls or housed with one or more familiar animals. During the physical introduction, the largest possible area, which had multiple escape routes, was provided. After the initial physical introduction, individuals were separated and either returned to their home stall or were housed with one or more familiar animals.

TABLE 4. Elephant behaviors by category*

Behavior category	Specific behavior	Behavioral definition
Active aggression	Head butt	Butting forehead or base of trunk against body or forehead of another.
	Tusk	Contact of tusk(s) with another elephant or object accompanied by a forward, lunging motion. Head of the initiator is usually held up.
	Charge	Rapid, forward lunging motion or rapid gait toward another with head held above shoulders, ears held perpendicular to the body and trunk held up. No physical contact.
	Push	Contacts another with the body, head or base of trunk causing other to move.
	Drive	Placing head or tusks to rear of another resulting in a continual displacement of at least two body lengths.
	Trunk hit	Quick sharp contact to another with the dorsal side of the distal end of the trunk.
	Spar	Individuals face each other head-to-head with raised chins, pulling and pushing with intertwined trunks. Includes mouth wrestling with trunks thrown back over the head. Head is held above shoulders. Also seen in play situations. See distinctions under Affiliative Behaviors.
	Push down on head	Pushing down on another's head with the base of trunk, tusks or open mouth.
	Kick	Kicking with forefoot or hind foot. May be observed in play. Distinction: in play it will be directed at objects.
	Throw object	Throwing an object toward a conspecific utilizing the trunk.
	Kick object	Kicking an object toward a conspecific.
	Chase	Rapid pursuit of a conspecific actively moving in a direction away from the initiator.
	Other active aggression	Any other active aggressive behavior.
	Passive aggression	Head shake
Head up/ears out		Head is held up above shoulders with trunk up and ears out perpendicular to the body. Ears may be waved. May be observed with Head shake. May be observed in play situations. Distinction: in play, it will be directed at objects.
Water spray		Expulsion of water previously drawn into the trunk in the direction of a conspecific.
Trunk over back		Placing at least two-thirds of length of the trunk over the back, head or neck of another. Does not include sweeping hay, sand or other material from another. This behavior may be confused with the affiliative variations. See Sweeping listed in Affiliative Behaviors for distinctions.
Mount		Raising the forelegs and placing them along the spine of another individual from the rear, as in copulation.
Snort		Rapid forcing of air from the trunk in a single short burst.
Displace		Initiator approaches recipient which moves from position. Position of recipient is then occupied by initiator within one body length. No physical contact.
Follow		Following within one body length of the other for at least two body lengths.

(continued)

TABLE 4. Continued

Submissive behavior	Other passive aggression Avoid	Any other passive aggressive behavior. Moving/orienting away from another animal that is either approaching or within trunk length.
Undesirable/ stress-related behavior	Back up Scream Pacing	Walking backward into another animal. High frequency vocalization emitted from the opened mouth (not from the trunk). Repetitive locomotor activity. "5 second rule."
	Head banging Rocking Trunk banging Other stereotypic behavior	Banging head against bars, doors or objects. "5 second rule." Standing in one position while rocking or swaying the body. "5 second rule." Repetitively striking bars, doors or objects with trunk. "5 second rule." Any other stereotypic behavior. "5 second rule."
Affiliative behavior	Trunk intertwine Play spar	Animals stand head-to-head, intertwine trunks. This behavior may be confused with trunk wrestling/sparring. Distinctions: less muscle tension in trunk, heads are not held above shoulders. "5 second rule." Individuals face each other head-to-head with raised chins, pulling and pushing with intertwined trunks. Includes mouth wrestling with trunks thrown back over the head. This behavior is also seen in aggressive situations. Distinctions: overall intensity level is less during play, heads may/may not be held above shoulders. "5 second rule."
	Sweeping	Placing two-thirds of the trunk over the back or head of another, with sweeping motions. This behavior may be confused with Trunk over back. Distinction: The aggressive version includes a higher degree of tension in the trunk tip. "5 second rule."
	Head rubbing Palatal pit area contact	Rubbing head, face or body against another. "5 second rule." Trunk tip touching the palatal pit area of another. "5 second rule."
	Temporal gland contact Genital contact	Trunk tip touching temporal gland openings of another. "5 second rule." Trunk tip touching genitals of another. "5 second rule."

*"5 second rule"—the behavior must be observed for at least 5 sec before being recorded and the behavior must stop for more than 5 sec before an additional occurrence is recorded.

As the physical introduction phase continued, the animals were housed together for increasingly longer time periods.

Protocol for fecal sample collection and corticoid analysis

Fecal samples were collected during each phase of both introductions. The elephants were individually housed at night. All fecal samples were collected daily between 0700 and 1000 hr, and were taken either from feces that were observed to be excreted or from the freshest sample available in the home stall. Fecal material was taken from at least three areas of the fecal bolus, placed in a 50-ml polypropylene centrifuge tube (Falcon Blue Max; BD Biosciences, Bedford, MA), and frozen at -20°C . Gloves were worn during collection to prevent sample contamination. Individual samples were lyophilized, pulverized, separated from undigested plant material, and shipped to the Conservation and Research Center in Front Royal, VA. Analysis for corticoid concentration was completed using a technique described by Graham and Brown [1996]. Approximately 0.2 g of fecal powder was extracted in an aqueous 90% ethanol solution. The extracted sample was then subjected to a double-antibody I-125 radioimmunoassay for corticoids (ICN Biomedical Inc., Costa Mesa, CA) and expressed in ng/g. Intra-assay coefficients of variation (CVs) were $<10\%$, and interassay CVs were $<15\%$.

Data Analysis

Data analysis consisted of a series of statistical procedures following methods described by Figueredo et al. [1992]. The first step was to perform a Multiple-P-Type factor analysis by first controlling for individual differences in behavior and then factor analyzing the behavioral measures to determine which behaviors co-occurred in time. This revealed the animals' behavioral states. Following this, the relationship of any behavioral states to subject, time, introduction type, and introduction phase was tested using the general linear model. This statistical approach is similar to an analysis of variance (ANOVA) except that it allows the use of continuous and categorical independent variables. For a detailed description of the analysis techniques used, see Burks [2002].

RESULTS

A total of 325 hr spent collecting behavioral data, and 181 fecal samples were recorded for the two introduction types between 2 September 1997 and 24 March 1998. See Table 5 for the number of hours spent collecting data and the number of fecal samples collected in each introduction phase for each introduction type. Variation in the number of hours spent collecting data and the number of samples in each introduction phase reflects the intuitive decision-making criteria used during the nonsequential introduction, and husbandry considerations during the sequential introduction period.

Behavioral Results

The factor loadings derived from the Multiple-P-Type factor analysis indicated that four of the behavioral categories measured were indicators of a single state (hereafter referred to as the "Social" factor), which explained most of the variance in

TABLE 5. Hours of data and number of fecal samples collected in each introduction phase during the two introduction types

Introduction type	Introduction phase	Hours of data	Fecal samples
Nonsequential	Baseline	53	15
	Visual contact	12	4
	Limited tactile contact	39	24
	Physical introduction	69	61
Sequential	Baseline	36	27
	Visual contact	38	18
	Limited tactile contact	51	20
	Physical introduction	27	12

Table 6. Factor loadings of behavior categories onto the social factor

Behavior category	Factor loading
Active aggression	.315
Passive aggression	.729
Submissive behavior	.330
Undesirable/stress related behavior	.125
Affiliative behavior	.643

those measures (see Table 6). It should be noted that while undesirable/stress-related behavior was indicative of the Social factor, it was not included in the factor solution because of low factor loading.

Role of affiliative behavior: hypothesis 1

The Social factor included the following behavioral categories: active aggression, passive aggression, submissive behavior, and affiliative behavior. Affiliative behavior loaded highly on the factor (.643), indicating that affiliative behavior was more indicative of the behavioral state observed than was expected (see Table 6). Although we had hypothesized that affiliative behavior would not vary systematically in either introduction type (hypothesis 1), the results indicated the importance of gathering data on these behaviors in order to obtain the most clear and complete explanation of the behavior patterns observed.

Behavior patterns between introductions: hypothesis 2

Of the four other behavior categories measured, only undesirable/stress-related behavior was not included in the factor solution because of low factor loading (.125) (see Table 6). All of the behaviors included in the factor were social in nature. To examine hypothesized differences in behavior patterns, a unit-weighted score for the Social factor (average frequency of the four behavior categories included in the factor) was computed for each individual observation period.

The general linear model indicated that the model (described by predictors of individual subject, time (the number of days from the beginning of the baseline

phase), introduction type, and introduction phase) accounted for 52% of the variance in the Social factor [$R^2 = .52$, $F(39, 286) = 7.98$; $P < .0001$].

The main effect of introduction type was significant [$F(1,286) = 28.36$; $P < .0129$], indicating differences in the frequencies of behaviors comprising the Social factor between the introduction types. The negative direction of the effect (regression parameter estimate: $b = -1.339$) indicated that fewer of the behaviors in the Social factor (active aggression, passive aggression, submissive behavior, and affiliative behavior) were observed during the sequential introduction. Additionally, there was a significant main effect of introduction phase [$F(1,286) = 20.33$; $P < .0064$], and the positive direction of this effect ($b = 4.501$) indicated that as the subjects progressed through the introduction phases, increases in the frequency of behaviors were observed, regardless of introduction type.

The interaction of time and introduction type was significant, indicating that the effect of the type of introduction varied with the progression of time [$F(1,286) = 15.16$; $P < .0300$]. The direction of this effect was positive ($b = .071$), indicating that the rate of increase in the behaviors comprising the Social factor (active and passive aggression, submissive behavior, and affiliative behavior) was greater in the sequential introduction. The second significant interaction effect was that of time and introduction phase [$F(1,286) = 23.75$; $P < .0046$]. This effect was negative ($b = -.019$), indicating that even though changes in the phases of introduction brought about increases in the frequency of behaviors, the frequencies decreased over time within each phase of introduction.

Taken together, these findings support the hypothesis that attenuation in the rates of behavior would be slower during the nonsequential introduction (hypothesis 2). Although increases in behavior were observed related to phase transitions in both types of introduction, behavior attenuated at a faster rate during the sequential introduction.

Role of undesirable/stress-related behavior: hypotheses 2 and 3

Because undesirable/stress-related behavior was not included in the factor solution, additional analyses using the general linear model were conducted to test the effects of the predictors on this behavioral category with and without the effect of the Social factor removed. A single significant interaction effect was determined: type of introduction by introduction phase (Social factor removed: [$F(1,285) = 15.31$; $P < .0297$]; Social factor not removed: [$F(1,286) = 13.67$; $P < .0343$]). These findings indicated significant differences in the effect of introduction phases between the introduction types. The direction of the effect was positive ($b = .48$), indicating that although there were no significant main effects of introduction type or introduction phase, there were larger increases in undesirable/stress-related behaviors between phases during the sequential introduction. However, the average frequency of undesirable/stress-related behaviors across subjects was the lowest for all categories with 1.09 (st.dev: 4.09) per hour in the nonsequential introduction, and 0.78 (st.dev: 3.33) per hour in the sequential introduction (see Table 7). These data did not support the hypotheses that this behavior category would show the same trends as agonistic behaviors (hypotheses 2 and 3).

TABLE 7. Average frequency per hour and standard deviation (in parentheses) of each behavior category in each introduction type

Introduction type	Active aggression	Passive aggression	Submissive behavior	Affiliative behavior	Stress-related behavior
Nonsequential	1.91(2.95)	14.51(12.13)	6.25(10.50)	5.06(7.39)	1.09(4.09)
Sequential	2.20(7.63)	12.19(8.91)	5.99(9.66)	3.44(6.01)	0.78(3.33)

Physiological Results

Analysis using the general linear model indicated that the model accounted for 59% of the variance in corticoid concentration [$F(39, 141) = 5.25$; $P < .0001$]. A single significant main effect was determined: type of introduction [$F(1, 141) = 15.72$; $P < .0287$]. This effect was negative ($b = -4.75$), indicating that lower overall levels of corticoids were measured during the sequential introduction. Additionally, there was a systematic change in corticoid levels within phases of introduction, as indicated by a significant interaction of time by introduction phase [$F(1, 285) = 9.31$; $P < .0284$]. The effect was negative (-0.10), indicating that corticoid levels decreased within each introduction phase. Finally, there was a significant interaction effect of time by introduction type by introduction phase [$F(1, 131) = 13.31$; $P < .0355$], and the positive direction of this effect ($b = 0.06$) indicated that the magnitude of change associated with sequential introduction was larger.

Overall, the data indicated that the nonsequential introduction elicited a larger physiological stress response. This supported hypothesis 3 (i.e., that fecal corticoid levels would be higher when a nonsequential introduction method was used). However, the data did not support the hypothesis that there would be either no decrease or a slower rate of attenuation of fecal corticoid concentrations during the nonsequential introduction (hypothesis 2). This indicated that although introductions are inherently stressful to elephants, the sequential method described by Burks et al. [2001] brought about a comparatively lower physiological stress response.

To examine the relationship between behavioral and corticoid results, correlations between residualized scores for hormone concentrations and each behavior category were computed. Since measures of glucocorticoids are often not specific to stressors alone [Mason and Mendl, 1993], this analysis allowed us to determine whether changes in fecal corticoid concentration complemented behavioral measures throughout the introduction process. The only behavior category that significantly correlated with fecal corticoid concentration was submissive behavior ($r = .250$; $P < 0.03$). Given that fecal corticoids did not show the same patterns of change in relation to the introduction model, it is not surprising that this measure was not correlated with other behaviors.

DISCUSSION

The primary goal of this study was to compare two introduction methods and to determine their effectiveness in assisting with day-to-day decision-making regarding elephant introductions. While the data indicated that aggression contributed to the overall behavior patterns observed, affiliative behavior also

made a surprising contribution to behavior patterns. Although only passive aggression had a higher factor loading than affiliative behavior, the results can be interpreted to signify that agonistic behavior was strongly influenced by the introduction method. With the exception of active aggression, which occurred in relatively low frequencies, there were lower rates of the other two agonistic categories (passive aggression and submissive behavior) during the sequential introduction (see Table 7).

Regardless of the type of introduction used, there was a significant increase in rates of behavior as the elephants were placed in increasingly closer proximity. The rates of these behaviors then decreased within each phase of introduction. These findings indicate that the different phases of introduction had similar effects, regardless of introduction type. These data alone may suggest that there is no overall benefit to using the sequential introduction method. However, the sequential introduction method resulted in lower rates of behaviors in the Social factor than the nonsequential method. Given the contribution of agonistic behavior categories (including active and passive aggression) to general behavior patterns, the results suggest that the sequential method of introduction did effectively manage aggression during these introductions. These findings closely match those described by Burks et al. [2001], and suggest that the general patterns observed are likely to be found in other applications of the sequential introduction method.

Undesirable/stress-related behaviors did not load onto the Social factor, were observed less often than all other behavioral categories, and did not show any major significant differences between the two types of introduction, indicating that this behavior category was a poor predictor of overall behavior patterns observed. In general, individuals did not exhibit behavioral responses to the stress of the introductions. However, the role of these behaviors may still be important in other introductions with elephants (particularly when males are involved) and other species. While the results of fecal corticoid analyses were not available to animal managers during day-to-day decision-making during this study, hormonal data did provide insight regarding differences in the effects of the two introduction types on stress in individuals. Overall, there were higher concentrations of corticoids measured during the nonsequential introduction. These results indicate that while all introductions are stressful to individuals, regardless of type, the subjects showed significantly less overall physiological responses during the sequential introduction.

Glucocorticoid excretion is influenced by numerous factors. Increases in excretion may be elicited by aversive stressors, but may also be brought about by events considered to be neutral or pleasant (for review see Mason and Mendl [1993]). In this study, corticoids correlated only with submissive behavior, suggesting that psychosocial stress influenced physiological response more so than affiliation, for which there was no correlation. Additionally, patterns of corticoid excretion were similar across introduction types, although lower concentrations were observed during the sequential introduction. The possibility exists that because the sequential introduction occurred after the nonsequential introduction, the subjects' physiological responses to the sequential introduction were suppressed due to habituation or other factors. Given that the two introduction sessions were separated by several months, and that similar response patterns were observed, it seems reasonable to

conclude that the sequential introduction method was directly related to lower corticoid concentration. Further investigation would strengthen this argument.

The method described by Burks et al. [2001] requires the development of a model that is valid, efficient, flexible, and applicable to a variety of contexts. The results of this study indicated that the sequential methodology for introductions successfully managed aggression and reduced the physiological stress response in the individuals involved.

From a Model to Day-to-Day Decision-Making

The key advantage of the methodology used is that data can be collected for immediate use in influencing the introduction plan. The data collection methods employed in this study can be easily and efficiently used in day-to-day decision-making. Behavioral data can be collected, summarized immediately, and used to assist in decision-making. Animal managers are often present during introduction periods, and can collect data while monitoring progress. These data could then be compared to the expected patterns of behavior and used to augment intuitive assessments during decision-making.

The results of this study, along with those of Burks et al. [2001], indicate general patterns of behavior that are likely to be observed in other introduction contexts. By collecting data for each individual and summarizing it for each behavioral category (e.g., not collapsed into a single category of social behavior), animal managers could obtain an instantaneous and objective assessment of each individual's behavior. While the interpretation of any data involves some level of subjectivity, empirically collected data can provide an objective tool with which to assess the behavior of the animals involved in the introduction. Managers should examine the data and wait for aggression levels that have increased during phase transitions to return to baseline levels before they move forward in the introduction. By doing so, the individual animals will be provided a means of expressing natural aggression in situations that limit the potential for wounding or other aggression that might hinder further integration into the social group.

Every introduction undertaken in zoos is subject to a host of factors that can influence success. These include everything from the social history of the animals involved to the experience of the staff and the design of the facility. It should be clearly stated that the methodology described here should never be used as a stand-alone tool in planning and implementing introductions. Rather, this methodology can serve as one tool in the "toolbox" available to animal managers. In addition to the factors listed above, the duration and intensity of aggression should be considered by animal managers during decision-making. Given that one must consider each introduction in a situation-specific context, intuitive assessment and empirical data can be used hand in hand to maximize success.

Inherent in these recommendations is the assumption that introductions should be planned in terms of days rather than hours. Attenuation of behavioral responses may occur within one introduction session (i.e., within an hour of limited tactile contact); however, animal managers should consider the use of a conservative approach, allowing more time for aggression to be expressed and then decrease. As the welfare of all individuals involved in the introduction must be considered, a conservative strategy should prove effective.

CONCLUSIONS

1. Regardless of the introduction method used, increases in behavior were observed as the animals progressed through phases of introduction, and then attenuated during those phases. This general pattern of behavior has been documented in other studies, and may be predictive for other introduction situations.

2. Affiliative behavior was more indicative of overall behavior patterns than expected, which highlights the importance of measuring these behaviors during introductions.

3. The use of a rigorous systematic protocol for introductions brought about lower overall behavior and fecal corticoid concentration compared to a less systematic protocol.

4. The use of a rigorous and systematic introduction protocol, and simple empirical methodologies, can be an effective tool for animal managers in managing aggression and minimizing stress during animal introductions.

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