INTRODUCTION OF FOREIGN FEMALE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) INTO AN EXISTING GROUP: BEHAVIOURAL REACTIONS AND CHANGES IN CORTISOL LEVELS

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The present study examined the extent to which the introduction of three female Asian elephants (aged 3, 11, and 27 years) into a group of 1.4 (1 male, 4 female) elephants at Münster zoo, Germany, affects the behaviour and urinary cortisol levels of the animals involved. At Münster, only the females were monitored — the bull was mainly kept separate. Behavioural observations were carried out before transfer and during the six-month period following transfer, and urine samples were collected regularly from each elephant during the whole observation period. All elephants showed behavioural changes to the process of introduction. The transferred animals increased their social behaviour after arrival in the foreign zoo. Two of them showed an increase in stereotypies and one a reduction in stereotypies. The elephants at Münster reacted with decreased frequencies of stereotypies and increased frequencies of social behaviour and manipulation/exploration behaviour. Six months after transfer, three of the four elephants at Münster and one of the three transferred elephants showed nearly the same behavioural activity pattern as before transfer. One female still showed elevated stereotypic behaviour. From the four elephants in which cortisol measurements could be reliably performed (two of the transferred elephants and two elephants at Münster), only one individual at Münster responded to the process of introduction with a short-term elevation in urinary cortisol levels. One elephant showed a negative correlation between locomotion and cortisol levels and one a positive correlation between stereotypies and cortisol levels. Taken together, the results suggest that transfer and introduction caused some stress responses in the elephants, but that stress was neither prolonged nor severe. Serious welfare problems may have been prevented through individual behavioural coping mechanisms and former experience with stressful situations.

Keywords: *animal welfare, Asian elephant, behaviour, stress, urinary cortisol*

Introduction

The Asian elephant has been listed as endangered by the Convention on International Trade in Endangered Species (CITES) since 1976. Because numbers in the wild have continued to decline progressively over the subsequent period, maintenance of a reproductively viable captive population represents an important component of the overall conservation strategy for this species. Correspondingly, the captive population of Asian elephants in Europe is managed by the European Endangered Species Programme (EEP) with the aim of increasing

reproductive output and establishing a self-sustaining demographically stable population (Tudge 1991; Dorresteyn & Belterman 1999).

To date, however, breeding success has been limited by, amongst other things, the fact that many zoos are not able to keep bulls, so animals with breeding potential may have to be transferred to other facilities for the purposes of breeding (Schmid 1998a). In this respect, transfer of adult bulls is problematic because of their requirement for special housing facilities; also, bulls in general are considerably more difficult to handle safely than are females. As a consequence, the transfer of females to groups containing a bull has become an integral part of the breeding management of Asian elephants in captivity. However, such transfer of females between groups is contrary to circumstances in the wild, in which sexually maturing males leave their family units, while females remain in their natal herds where they establish strong social relationships (Sukumar 1994). Although the transfer of females represents the most practical option, the extent to which loss of familiar environment, transport, and integration into a group of foreign animals represent stressful situations for the elephants is not known. Assessment of this is important, however, because in addition to its implications for welfare, stress would itself be counterproductive to the objective of successful breeding.

In general, stress is viewed as a biological and functional response to environmental and bodily demands (Bohus *et al* 1987). Stress elicits behavioural and physiological reactions in animals and is often combined with elements of conflict behaviour such as displacement activities, redirected activities, and/or stereotypies (Ödberg 1987; Wiepkema 1987; Jensen & Toates 1997). However, Ödberg (1987) emphasised that some animals do not show any of these behaviours, and accurate measurements of all activities are required in order to detect behavioural responses to stressful situations.

On a physiological level, stress is generally associated with enhanced adrenal activity and a corresponding elevation in levels of glucocorticoid hormones such as cortisol (Henry & Stephens 1977; Ladewig 1987; Broom & Johnson 1993). Increased cortisol levels have been observed in a variety of species subjected to potentially stressful circumstances, such as after transport of okapis (Schwarzenberger *et al* 1998), after aggressive interactions between spotted hyenas (Goymann *et al* 1998), after restraining experiments with cheetahs (Jurke *et al* 1997), and during separation from social partners in sheep and squirrel monkeys (Mendoza *et al* 1978; Parrott & Thornton 1988). Measurement of cortisol levels may also be used for the assessment of stress in elephants. Elevated cortisol levels have been observed during introduction of one female into an existing herd (Dathe *et al* 1992), during parturition (Brown & Lehnhardt 1995), and after adrenocorticotrophin (ACTH) challenge (Brown *et al* 1995).

Behavioural and physiological responses to stressful events show great individual differences. They are influenced by ontogenetic processes, learning, former experiences and social relationships (Henry & Stephens 1977; Rushen 1986; Wiepkema 1987; von Holst 1994; Jensen & Toates 1997; Jurke *et al* 1997). Moreover, the behaviour of an animal can influence its physiological stress reaction. For example, in some cases, stereotypies have been shown to reduce the stress response (Ödberg 1987; Kennes & Rycke 1988; Schouten & Wiepkema 1991), although other investigations have yielded conflicting results (Dantzer *et al* 1987; Borell & Hurnik 1991; Dantzer 1991; Schouten *et al* 1991; Terlouw *et al* 1991).

In the present study, we investigated the introduction of three female elephants into a foreign group in a distant zoo. Using a combined behavioural and physiological approach, the study aimed to determine whether transfer and introduction cause stress to the animals

concerned and whether such stress is associated with the occurrence of special behavioural activities (eg stereotypies) and possible elevation in urinary cortisol levels. It is hoped that the outcome of this study may yield useful information for elephant breeding management.

Materials and methods

Animals and keeping conditions

Three female Asian elephants, Kanaudi and Ratna from Tierpark Hagenbeck (Hamburg, Germany) and Bernhardine from Diergaarde Blijdorp (Rotterdam, Netherlands), were transferred to the Allwetterzoo Münster (Germany) in October 1995. Kanaudi and Ratna arrived on the 4th and Bernhardine on the 30th of October. Age at time of transfer, origin of the elephants, date of arrival at Münster and former keeping locations are listed in Table 1. Bernhardine and Ratna were transferred to Münster for breeding. Kanaudi, who had a close relationship with Ratna, was transferred together with Ratna to support and take care of her in the foreign group. All three transferred elephants, as well as the four female elephants already kept at Münster, were well trained and were handled in a hands-on management system in which the keepers handle the elephants directly and share the same unrestricted space with the elephants (Otten 1994; Adler 1996; Schmid 1998b).

Table 1 **Individual data for the elephants. Name, age, origin, date of arrival at Münster, and former keeping locations are given (i) for the transferred elephants (ii) and for the elephants at Münster.**

Name (abbreviation)	Age[a]	Origin	Arrival at Münster	Former keeping locations
(i) Transferred animals				
Kanaudi (K)	27	Wild (India)	4.10.95	1974–95 Hamburg, Germany
Ratna (Rt)	3	Zoo born (1992)	4.10.95	1992–95 Hamburg, Germany
Bernhardine (B)	11	Zoo born (1984)	30.10.95	1984–88 Rotterdam, Netherlands 1988 Krefeld, Germany 1988–95 Rotterdam, Netherlands
(ii) Animals at Münster				
Tefi (T)	29	Unknown	1974	Unknown
Rada (Rd)	28	Unknown	1983	?–1983 Circus Royal
Thong Thai (TT)	7	Vietnam	1994	1988–94 Working camp, Vietnam
Praya (P)	19	Unknown	1995	1979–98 Cottbus, Germany

[a] Age in years at time of transfer (October 1995).

Ratna was born at Hamburg and kept together with her mother unshackled in a box during the night and bad weather conditions (Table 2). Kanaudi and Ratna were kept together with 10 other females and one three-year-old bull in a herd. Bernhardine was born at Rotterdam and reared by her mother. At the time of transfer, she lived together with five females including her mother and two younger sisters plus a six-year-old male in a herd. Two adult females and an adult bull were kept separately. The four female elephants at Münster — Tefi, Rada, Praya, and Thong Thai — were kept together in one herd. The matriarch of the group was Rada. The 17-year-old bull, Alexander, was mainly kept separate. Occasionally, he stood together with the female herd for a few hours to encourage breeding. More detailed information about the keeping conditions in the three zoos is given in Table 2.

Table 2 Keeping conditions in the zoos.

	Hamburg	Rotterdam	Münster
1. Group size[a]	2.12	2.8	1.4
2. Outdoor enclosure			
Space	3000 m²	3000 m²	2700 m²
Enrichment	Bath, sand, rubbing places, wallow	Bath, sand, rubbing places, wallow	Bath, sand, rubbing places, loam
3. Indoor enclosure			
Space	Individual boxes, each 25 m²	500 m²	500 m²
Enrichment	No enrichment	Bath, rubbing places	Bath, rubbing places
4. Housing			
Night	Shackled in boxes, calves unshackled	Unshackled in the group in indoor enclosure	Unshackled in the group in indoor enclosure
Day — bad weather	Unshackled in boxes	Unshackled in the group in indoor enclosure	Unshackled in the group in indoor enclosure
Day — good weather	In the group in outdoor enclosure	In the group in outdoor enclosure	In the group in outdoor enclosure
Shackled keeping	During the night 1900h–0830h	No shackled keeping	Irregularly for handling or training
Keeping of adult bulls	Separate, sometimes together with some females	Separate	Separate, sometimes with female herd
5. Man–elephant contact			
Management system	Hands on	Hands on	Hands on
Occupation	Training, performances, walks outside the enclosures	Training, performances	Training, performances
Public feeding	The whole day in indoor and outdoor enclosure	Not permitted	Permitted at fixed times and special places in indoor and outdoor enclosures

[a] Number of males.number of females.

Procedure of transport and introduction

All three elephants were transported in wooden boxes on lorries and two or three legs were chained during transport. Before boxing, Kanaudi and Ratna were slightly sedated. Bernhardine was transported without any sedation. At Münster, all three females left the boxes without force; they obeyed the command given by familiar keepers without any problems. In total, boxing and transport took seven hours for Bernhardine and 10 hours for Ratna and Kanaudi.

During the first two days after their arrival at Münster, contact between Kanaudi, Ratna and the group at Münster mainly took place through barriers. The elephants were kept together for only a short period of time (30–60 min per day) without any barriers. All contact between the animals during this first phase took place in the presence of keepers. If the animals started to interact with serious aggression (eg pushing, trunk-whipping, biting,

kicking, attacking), the keepers intervened and called them back. After the first two days, Kanaudi and Ratna spent the whole day together with the herd. During the night they were separated from the herd by barriers. Contact between the animals was possible through the barriers.

Because Bernhardine showed aggressive behaviour (threatening, trunk-whipping) against the other elephants during their first contacts through the barriers, she was kept separately during the first three weeks. Once, four days after her arrival, she was allowed to join Kanaudi and Ratna in the indoor enclosure for one hour, a meeting that resulted in aggressive head-to-head fighting between her and Kanaudi. After three weeks of single housing, during which Bernhardine had contact with the other elephants only through barriers, her introduction was started again. For two days, she spent several hours together with the rest of the group in the presence of the keepers. The keepers intervened in aggressive interactions between the animals. Afterwards, Bernhardine was housed with the group for the whole day and no serious problems occurred. During the night, she was separated in a box without any possibility of contact with the other animals.

Behavioural observations

The behaviour of the animals was observed during eight observation periods from June 1995 to April 1996 (Table 3). During the first observation periods (R, H, M1), the behaviour of the transferred animals at Hamburg (H) and Rotterdam (R) and the animals already at Münster (M) was recorded before any transfer took place (Table 3). The integrations of Kanaudi, Ratna, and Bernhardine into the group at Münster were observed over five observation periods for Kanaudi and Ratna (M2, M3, M4, M5, M6) and four observation periods for Bernhardine (M3, M4, M5, M6), respectively, until April 1996. The different observation periods lasted between six and 19 days (Table 3).

Table 3 Observation periods. For each observation period, date, days of observation, total number of recorded scans, situation and the observed animals are shown.

Zoo	Observation period	Date (days of observation)	Total number of scans	Situation	Observed animals[1]
Rotterdam	R	June 1995 (16)	510	Before transfer	B
Hamburg	H	July 1995 (19)	1463	Before transfer	K, Rt
Münster	M1	Sept 1995 (6)	1187	Before transfer	Rd, T, P, TT
Münster	M2	Oct 1995 (9)	1924	Arrival/introduction of K, Rt	Rd, T, P, TT, K, Rt
Münster	M3	Nov 1995 (10)	3204	Arrival of B	Rd, T, P, TT, K, Rt, B
Münster	M4	Dec 1995 (9)	2311	Introduction of B	Rd, T, P, TT, K, Rt, B
Münster	M5	Jan 1996 (9)	2689	—	Rd, T, P, TT, K, Rt, B
Münster	M6	April 1996 (10)	3310	—	Rd, T, P, TT, K, Rt, B

See Table 1 for abbreviations for elephants.

During all observation periods, eight different behavioural categories were recorded every 10 minutes by scan-sampling (Martin & Bateson 1993). Behavioural elements recorded in the different categories are listed in Table 4. Observations started in the morning when the keepers began their work (about 0800h) and ended in the evening when the keepers finished work (about 1800h). The number of scans carried out during the different observation periods varied with the number of observation days and the number of observed animals (Table 3).

Table 4 Elements classified in different behavioural categories.

Category (abbreviation)	Elements
Stereotypies (ste)	Weaving
	Stereotyped locomotion
Social behaviour (soc)	Elephant contacts the body of another elephant with trunk, head, foot or other part of the body
	Rubbing bodies against each other
	Smelling any part of another elephant's body
	Climbing or mounting onto another elephant
Comfort behaviour (com)	Picking up and throwing substrate (sand, food, water) onto the body
	Rubbing against objects
	Scratching with parts of the body or with objects
	Wallowing
	Excreting urine or faeces
Feeding (fee)	Preparing, picking up and eating food
	Sucking and drinking water
	Suckling milk
Locomotion (loc)	All forms of walking
	Climbing
Resting (res)	Resting in standing or recumbent position
	Sleeping in standing or recumbent position
Manipulation/exploration (mex)	All forms of manipulating objects or substrate with trunk, foot, head or other part of the body (picking up, kicking, throwing, breaking)
	Smelling objects
	Scenting
Others (oth)	Elements that cannot be classified in any mentioned category

Cortisol analyses

At least ten urine samples were collected from all animals (transferred elephants and elephants at Münster) 1–2 months before transfer. One week before and one week after a transfer took place, samples were taken daily from all study females. Afterwards, weekly urine samples were collected from all animals until the end of the study period. All samples were taken within a two-hour period in the morning (0830h–1030h) in order to reduce the potential effect of diurnal variation in cortisol excretion, and stored frozen in polypropylene tubes until analysis. Urine was obtained by midstream catch.

Immunoreactive cortisol in urine samples was measured by enzyme immunoassay using an antiserum against cortisol-3-CMO-BSA (AB 1002, BioClinical Services, Cardiff, UK) and biotinylated cortisol as enzyme conjugate (Palme & Möstl 1997). For cortisol analysis, urine samples (100 µl) were diluted with 400 µl distilled water and extracted with 2 ml dichloromethane by vortexing for 10 min (Brown & Lehnhardt 1995). Samples were centrifuged at 2000 g for 5 min, the dichloromethane phase removed, dried under a stream of nitrogen at 45°C and finally reconstituted in 500 µl phosphate buffered saline (PBS; pH 7.2). Extraction efficiency, monitored by the recovery of 5000 counts per minute (cpm) of ^{3}H-cortisol added to each sample prior to extraction, was > 85 per cent.

For hormone analysis, extracted samples (50 µl) and cortisol standards (50 µl, 0.3–40 pg well^{-1}) were combined with labelled cortisol (50 µl) and antiserum (50 µl) and incubated overnight at 4°C. After incubation, the plates were washed four times, 150 µl (20 ng) streptavidin-peroxidase (No. S 5512, Sigma Chemie, Deisenhofen, Germany) in PBS buffer added to each well, the plates incubated at room temperature (RT) in the dark for 30 min and then washed again four times. Substrate solution (150 µl, including 0.025% tetramethylbenzidine and 0.05% H_2O_2) was added and the plates incubated at RT in the dark

for another 45–60 min. The enzyme reaction was stopped by the addition of 50 µl of 2M H_2SO_4 and absorbance measured at 450 nm. Sensitivity of the assay at 90 per cent binding was 0.5 pg. Intra- and inter-assay coefficients of variation of high and low value quality controls were < 10 per cent and < 13 per cent, respectively.

To control for the variations in the volume and concentration of the voided urine, the creatinine content of each urine sample was determined automatically by a creatinine analyser (Beckman Instruments, Brea, USA) using 1:10 diluted urine samples. Accordingly, urinary cortisol concentrations are expressed as ng mg^{-1} creatinine (Cr).

Treatment of data and statistical procedures

The proportion of scans in which a behavioural category was shown was calculated for each elephant and each observation period (number of scans in which behavioural category was shown divided by total number of scans recorded for each animal). Behavioural changes between different observation periods were analysed using *G*-Test and Extension of Fisher Test.

Urine cortisol levels before transfer were compared with levels after transfer for each elephant using Wilcoxon Signed Ranks Test. To analyse any correlations between behaviour and cortisol levels, Spearman rank correlation coefficients were calculated between cortisol levels on each day and proportions of scans in which each behavioural category was shown on the previous day.

Keeping conditions during the different observation periods were determined by the different percentages of time spent by the elephants in the indoor enclosure, in the outdoor enclosure, or shackled. Taking into account that changes in keeping conditions could influence the behaviour of the animals independently from any introduction aspects, the proportions of time spent in the various keeping conditions were correlated with the proportion of scans in which a behavioural category was shown for each elephant. If several tests were calculated with the same samples, levels of significance ($P = 0.05$) were corrected using the method of Bonferroni (Engel 1997).

Results

Behavioural observations

Short-term behavioural changes associated with introduction

Table 5 lists the results of behavioural observations during the different observation periods for the transferred animals. All three transferred animals showed a significant increase in frequency of social behaviour during the period of introduction (observation period M2 for Kanaudi and Ratna, observation period M4 for Bernhardine) compared with the preceding observation period (H for Kanaudi and Ratna, M3 for Bernhardine; Table 5). Additionally, Kanaudi and Ratna reacted with a significant increase in the category 'others' during introduction (Table 5). The arrival and introduction at Münster was combined with a significant increase in stereotypies for Kanaudi but not for Ratna, who showed significantly lower frequencies of stereotypies during arrival and introduction compared with the period before transfer. Bernhardine showed a significant increase in stereotypies during the period of arrival but a significant decrease during the period of introduction. Kanaudi and Bernhardine decreased their feeding activity when they arrived at Münster. Other significant changes during introduction were an increase in locomotory activity for Kanaudi and an increase in manipulation/exploration behaviour for Ratna (Table 5). In all other behavioural categories, the transferred animals showed no significant changes.

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Table 5 **Percentage of scans during which the transferred elephants performed various behavioural activities.**

Observation period	Situation	Behaviour Scans % ste[1]	soc	com	fee	loc	res	mex	oth
1. Kanaudi									
H	Before transfer	3	2	7	64	12	0	9	3
M2	Arrival/intro K, Rt	16*	6*	6	27*	18*	0	12	22*
M3	Arrival B	19	4	2*	48*	13	0	14	8*
M4	Introduction B	20	6	2	39*	15	0	16	11
M5	—	12*	4	0*	67*	10	1	7*	9
M6	—	7*†	2	7*	33*†	12	1	23*†	20*†
2. Ratna									
H	Before transfer	29	2	3	29	32	0	9	4
M2	Arrival/intro K, Rt	10*	5*	1	25	32	0	14*	16*
M3	Arrival B	23*	5	3	43*	25	0	10	5*
M4	Introduction B	15*	8	1	38	28	0	18*	4
M5	—	9*	3*	2	69*	31	0	4*	4
M6	—	3*†	4	4	33*	32	0	22*†	10*†
3. Bernhardine									
R	Before transfer	7	9	2	47	11	1	17	9
M3	Arrival B	39*	5	1	39*	9	0	12	7
M4	Introduction B	10*	12*	1	49*	12	0	15	8
M5	—	10	8	2	71*	7	0	7*	4*
M6	—	8	7	5*†	42*	15*	0	18*	7

[1] See Table 4 for abbreviations for behavioural categories.
* Significantly different (*G*-Test, Fisher Test) from the preceding observation period.
† Significantly different (*G*-Test, Fisher Test) from the situation before transfer (observation periods H and R).

The behavioural reactions of the elephants at Münster on the introduction of foreign animals are shown in Table 6. Tefi, Rada and Praya (but not Thong Thai) showed significant increases in the category manipulation/exploration during introduction of Kanaudi and Ratna (observation period M2; Table 6) compared with the preceding observation period. When Bernhardine was introduced (observation period M4; Table 6), all four animals at Münster showed an increase in manipulation/exploration behaviour compared with the preceding observation period, and these changes were significant for Thong Thai, Praya and Rada.

Of the animals at Münster, only Praya and Thong Thai frequently showed stereotypies. Tefi had no stereotypies and Rada showed her stereotypy only very occasionally. During the introduction of Kanaudi, Ratna and Bernhardine (observation period M2 and M4; Table 6), Praya and Thong Thai showed a significant decrease in their stereotypies compared with the preceding observation period (Table 6). Additionally, these two animals showed a significant increase in their social behaviour during introduction of the three foreign animals (Table 6). Rada showed a significant increase in social behaviour only during the introduction of Bernhardine (Table 6). Tefi showed no reaction in her social activities either during the introduction of Ratna and Kanaudi or during the introduction of Bernhardine. During the period in which Bernhardine was introduced (M4), all four animals at Münster showed a decrease in their feeding activities, which was significant for Tefi, Rada and Praya (Table 6).

Table 6 **Percentage of scans during which the elephants at Münster performed various behavioural activities.**

Observation period	Situation	Behaviour Scans % ste[1]	soc	com	fee	loc	res	mex	oth
1. Tefi									
M1	Before transfer	0	2	8	33	9	4	28	17
M2	Arrival/intro K, Rt	0	2	8	28	10	4	39*	12
M3	Arrival B	0	3	2*	50*	7	6	15*	19*
M4	Introduction B	0	3	3	40*	12*	3	19	22
M5	—	0	3	1	69*	6*	2	10*	11*
M6	—	0	1	11*	34*	8	4	24*	20*
2. Rada									
M1	Before transfer	0	3	9	30	17	4	15	23
M2	Arrival/intro K, Rt	1	3	10	31	11	5	26*	16*
M3	Arrival B	1	4	3*	51*	9	2*	10*	22
M4	Introduction B	0	10*	2	41*	18*	1	15*	17
M5	—	0	6	1	67*	13	3	4*	12
M6	—	0	5	16*†	35*	16	2	13*	17
3. Thong Thai									
M1	Before transfer	8	4	5	30	18	1	16	19
M2	Arrival/intro K, Rt	4*	9*	1*	30	18	0	17	22
M3	Arrival B	12*	8	1	47*	13	0	9*	14*
M4	Introduction B	0*	13*	1	42	18*	0	20*	10
M5	—	0	7*	1	69*	10*	0	9*	9
M6	—	2*†	5	5*	35*	21*	0	21*	15*
4. Praya									
M1	Before transfer	34	1	5	31	11	0	9	15
M2	Arrival/intro K, Rt	10*	10*	6	26	9	1	27*	12
M3	Arrival B	23*	5*	2*	47*	9	1	5*	12
M4	Introduction B	7*	13*	3	39*	12	0	14*	15
M5	—	13*	4*	2	39	7*	1	2*	10*
M6	—	20*†	4†	7*	36	9	1	20*†	10

[1] See Table 4 for abbreviations for behavioural categories.
* Significantly different (*G*-Test, Fisher Test) from the preceding observation period.
† Significantly different (*G*-Test, Fisher-Test) from the situation before transfer (observation period M1).

Long-term behavioural changes

In order to analyse the long-term effects of introduction on behaviour, the behavioural activities of the elephants before transfer were compared with their activities during the final observation period, which was about six months after the introductions took place (observation period M6; Tables 5 and 6). Of the transferred animals, Bernhardine showed significantly higher frequencies of comfort behaviour during the last observation period at Münster compared with the period before transfer while she was at Rotterdam (Table 5). She did not show significant changes in any other behavioural categories.

Kanaudi and Ratna showed significantly more manipulation/exploration activity and more behaviours belonging to the category 'others' after half a year at Münster (observation period M6; Table 5) compared with Hamburg (observation period H; Table 5). Kanaudi decreased her feeding activities and increased her stereotypies significantly, whereas Ratna showed a significant decrease in stereotypies (Table 5). In all other behavioural categories, neither elephant showed significant changes six months after their arrival at Münster compared with the period before transfer.

Of the animals at Münster, Tefi showed no significant changes in her behavioural activities six months after Kanaudi and Ratna arrived at Münster (observation period M6; Table 6) compared to before their transfer (observation period M1; Table 6). Rada showed a significant increase only in the category of comfort behaviour (Table 6). The reduction in Thong Thai's stereotypies that occurred during the introduction of the foreign animals was still evident during the last observation period (M6), and at M6 the frequency of stereotypies was significantly different from that during the period before transfer (Table 6). Praya increased her manipulation/exploration activities and social behaviour and decreased her stereotypies significantly. This corresponds to her reaction during the periods when the foreign animals were introduced (Table 6).

All observed behavioural changes, short- and long-term, showed no correlation with the time spent by the elephants in different keeping systems, indicating that these differences in keeping did not influence the observed changes in behaviour.

Cortisol analyses

Urinary cortisol concentrations could only be determined for four of the seven elephants — Kanaudi and Bernhardine (two of the three transferred elephants), and Tefi and Thong Thai (at Münster) — because, in virtually all samples from the other three elephants, creatinine levels were < 0.05mg ml^{-1}, preventing their use for indexing hormone concentration.

Neither Kanaudi nor Bernhardine showed a marked increase in urinary cortisol concentration after arrival at Münster. However, the first sample from Bernhardine after her arrival at Münster could be taken only on the second day. Seven days after Bernhardine's arrival, on day 78, Kanaudi showed a slight increase in her urinary cortisol levels (see Figure 1), which may have corresponded to an aggressive encounter with Bernhardine three days before.

Of the two elephants at Münster, Tefi showed a clear increase in cortisol levels after the arrival of foreign animals — two days after the arrival of Kanaudi and Ratna, and one day after the arrival of Bernhardine (Figure 1). In contrast, Thong Thai showed no obvious change in cortisol levels immediately after the arrival either of Kanaudi and Ratna, or of Bernhardine (Figure 1).

Comparison of cortisol concentrations before transfer with cortisol concentrations 260 days after transfer (Figure 1) showed no significant differences for Tefi ($median_{before}$ = 118.6 ng mg^{-1} Cr; $median_{after}$ = 118.4 ng mg^{-1} Cr; $P = 0.31$), Thong Thai ($median_{before}$ = 107.9 ng mg^{-1} Cr; $median_{after}$ = 90.6 ng mg^{-1} Cr; $P = 0.29$), or Kanaudi ($median_{before}$ = 73.9 ng mg^{-1} Cr; $median_{after}$ = 81.8 ng mg^{-1} Cr; $P = 0.64$). Levels of cortisol in Bernhardine were significantly lower after her transfer to Münster than they were while she was at Rotterdam ($median_{before}$ = 122.6 ng mg^{-1} Cr; $median_{after}$ = 74.3 ng mg^{-1} Cr; $P = 0.03$).

Correlations between behaviour and cortisol concentrations were found for Bernhardine and Kanaudi. Kanaudi showed a negative correlation between cortisol levels and locomotory activities recorded on the preceding day ($r = -0.8$; $P < 0.01$). Bernhardine's stereotypies were positively correlated with her urinary cortisol concentrations measured on the following day ($r = 0.9$; $P < 0.01$).

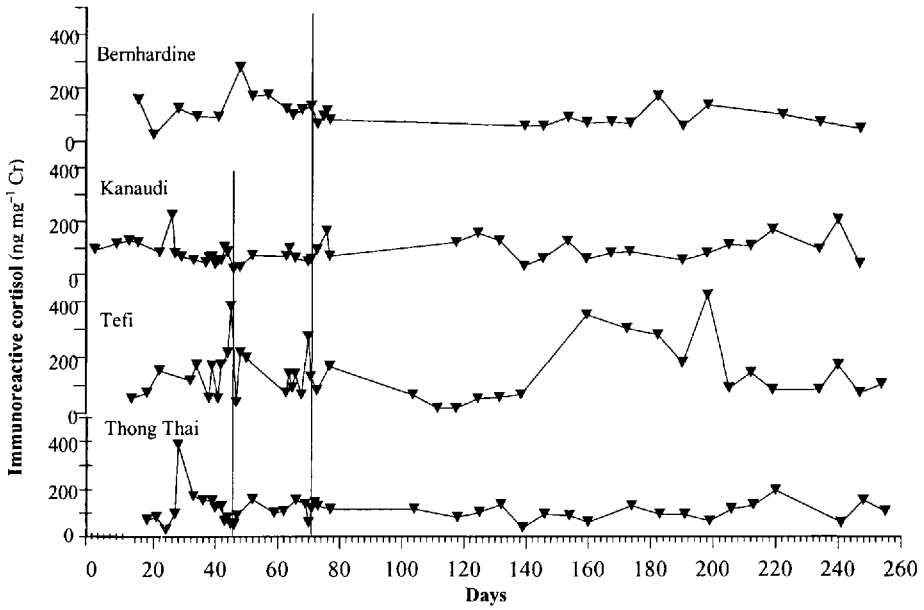

Figure 1 **Urinary cortisol levels of Kanaudi, Bernhardine, Tefi and Thong Thai from day 1 (21.08.95) until day 260 (06.05.96). On day 45 (04.10.95), Kanaudi and Ratna arrived at Münster. On day 71 (30.10.95), Bernhardine arrived.**

Discussion

Breeding management for captive Asian elephants generally requires transfer of females between groups rather than movement of bulls. Because this is contrary to circumstances in the wild, where females remain in their family groups throughout their lives, we have investigated whether such captive transfer procedures cause stress to the elephants concerned. Here, we report the effects of transfer and introduction on the behaviour and urinary cortisol levels of three transferred females and the members of a recipient herd at Münster.

All elephants that were observed showed behavioural changes in response to the process of introduction, but only a few of these would normally be interpreted as potential indicators of stress. One behavioural pattern associated with stress is the performance of stereotypies (Ödberg 1987; Wiepkema 1987; Jensen & Toates 1997). Only two transferred elephants, Kanaudi and Bernhardine, showed increased activities in stereotypic behaviour in response to the process of introduction. In the third transferred elephant, Ratna, the incidence of stereotypies was lower after her arrival than it was while in her natal group. One potential reason for this change in her behaviour could be the separation from her mother, who repeatedly showed aggressive behaviour towards her, including physical attacks. Thong Thai and Praya, the two elephants at Münster that showed relatively high frequencies of stereotypies while in their familar group, reduced their stereotypic behaviour after the arrival of the foreign elephants. As performance of stereotypies is often observed in situations in which animals are confronted with unvarying stimuli (Mason 1991; Wemelsfelder 1993), introduction of foreign animals may, under certain circumstances, represent a welcome

variation in the daily routine by providing novel stimuli and contributing to a more enriched environment.

Another behavioural sign of stress is frequent performance of displacement activities (Wiepkema 1987; Jensen & Toates 1997). Elements of displacement activities in elephants are included in manipulation and exploration behaviours (eg collecting sand in the trunk or manipulating objects such as sticks or stones). All elephants at Münster performed more manipulation/exploration behaviour during the period of introduction of the foreign animals. Of the transferred elephants, only Ratna reacted with increased manipulation/exploration behaviour than during the pre-introduction phase. Although the elevation in this behaviour type could be interpreted as representing increased displacement activity and thus as a behavioural stress reaction, it may also represent a response to other factors such as novel smells and sounds without a stress component.

All the other observed behavioural changes reported here are not associated with stress. Increase in social behaviour after introduction might result from increased exchange of social contacts while becoming acquainted with new companions. Changes in feeding activities are primarily influenced by feeding management rather than by the introduction process itself. Thus, of the parameters examined, only the increase in stereotypies shown by Kanaudi and Bernhardine could be interpreted as a behavioural sign of a stressful situation. Six months after transfer, Kanaudi still showed this increase, whereas the frequency of such behaviour in Bernhardine had returned to that shown at her previous location. The maintenance of increased stereotypic behaviour in Kanaudi might result from stress attributable to social conflicts caused by the proximity in Kanaudi's age to that of the matriarch of the group, Rada.

Investigation of long-term changes indicated that four elephants — one transferred (Bernhardine), and three already at Münster (Rada, Tefi, Thong Thai) — showed almost the same behavioural activity pattern six months after transfer as they did during the period before transfer, despite the change in environment. This would suggest, in the study described here, that transfer and introduction produced only short-term behavioural changes and that each animal had an individual specific behavioural activity pattern that was performed independently of their environmental situation. That environmental factors had little influence on the behaviour of the elephants in this study is also suggested by the lack of correlation between changes in keeping conditions and behaviour.

Data on cortisol excretion were unfortunately limited to four elephants, as creatinine levels in nearly all urine samples from the other females were below the detection limit of the assay. Further investigations would therefore be necessary to confirm the preliminary findings reported here. The results showed that cortisol levels were not consistent for individual elephants, and there was no clear indication that transfer and introduction resulted in elevated glucocorticoid output. Only one animal at Münster (Tefi) showed a short-term increase in urinary cortisol following introduction of the foreign animals, while all other females showed no clear changes in cortisol levels. The slight cortisol response of Kanaudi at day 78, seven days after the arrival of Bernhardine, was likely to have been attributable to heightened aggression between her and Bernhardine starting with the head-to-head fight on day 75 when they were kept together without barriers for one hour.

In the long-term, cortisol levels remained the same in three of the females (Tefi, Thong Thai, Kanaudi), while one transferred female (Bernhardine) showed a significant decrease. This suggests that although Bernhardine was confronted with a new environment (new enclosures, foreign elephants and keepers), she responded positively to the change instead of

perceiving her new environment as stressful. Although differences in handling procedures may have contributed to these changes, there is no direct evidence to support this. Thus, the potential behavioural signs of stress as indicated by the increased frequency of stereotypies for Kanaudi and Bernhardine could not be confirmed by elevated cortisol levels.

Together, the hormonal and behavioural data give no clear indication that the transfer and introduction process caused stress to the elephants beyond the short-term adjustment that is required to adapt to new management conditions and environment. Kanaudi was the only animal who showed a long-term behavioural stress response (increased stereotypic behaviour), but this stress response could not be confirmed by elevated adrenal activity. The findings of this study thus suggest that withdrawal of established social relationships, transport, and integration into a foreign group do not necessarily represent unduly stressful situations for elephants. To some extent, however, the elephants may have perceived stress but found ways to cope with this. Performance of stereotypies can be used to cope with stress (Kennes & Rycke 1988; Schouten & Wiepkema 1991). Ödberg (1987) suggested that animals that do not perform stereotypies experience stressful situations as much as do animals with stereotypies but are unable to develop stereotypies as coping mechanisms. This incapacity leaves them in even greater distress and adrenal activity becomes elevated. The finding that Tefi, who did not perform stereotypies, showed a short-term increase in urinary cortisol following introduction whereas the other three elephants (Kanaudi, Bernhardine, Thong Thai), who did not show elevated urinary cortisol levels, performed stereotypies frequently would be in agreement with Ödberg's hypothesis. The proposal that female elephants may use stereotypies to cope with potentially stressful situations is supported by the increased frequencies of stereotypic behaviour shown by Bernhardine and Kanaudi after their arrival at Münster, and by the positive correlation between levels of stereotypies and cortisol levels found for Bernhardine.

Another explanation for the lack of physiological stress reactions in most of our study animals might be their former experience with stressful situations; such former experience may have prevented elevated glucocorticoid output in response to the transfer procedure described here. Stress reactions are individually different. Investigations in pigs and guinea pigs have shown that an animal's subjective experience of a former stressful event influences its subsequent physiological stress reactions (Dantzer & Mormède 1981; Sachser & Lick 1991). Furthermore, it has been shown that frequent stress responses increase the resistance to stress (Ladewig 1987). All three of the elephants that did not show elevated urinary cortisol levels in response to introduction were accustomed to being confronted with new stimuli or potentially stressful situations. They were well trained, took walks outside their enclosures, had transport experiences, or had even changed keeping-location. On the other hand, Tefi, who showed a physiological stress reaction to introduction, had only limited experience with novel, potentially stressful situations. At the time of being confronted with the foreign elephants, she had lived at Münster for 24 years and her environment had not changed markedly over much of that time.

Bernhardine and Ratna were transferred to Münster for breeding. In January 1999, Bernhardine gave birth to a female calf, which unfortunately died one day after birth. Ratna was not sexually mature at time of transfer and as yet she has not been mated successfully.

Animal welfare implications

Our results show that transfer and introduction of female elephants into a foreign group does not necessarily represent an unduly stressful situation, either for the transferred animal or for the members of the group into which the elephant is introduced. Some stress, however, might

have been perceived by the animals, although it was neither prolonged nor severe. Behavioural coping mechanisms and former experiences of stressful situations seem to have prevented severe stress reactions. Therefore, potential transfer-elephants should be given experience of new stimuli and stressful events. This can be achieved using variable training, in which elephants have to continuously learn new tasks and be confronted with new situations (eg taking walks outside the enclosure, or working with foreign objects or other species). Furthermore, elephants should be exposed to new stimuli by presenting a variety of behavioural enrichment schemes within the enclosure, such as the introduction of new objects for playing or of unfamiliar foods that perhaps need special forms of manipulation before they can be eaten. In this way, elephants are likely to gain experience of unusual situations and, furthermore, to develop coping mechanisms that might help to prevent severe stress responses to potentially stressful events such as transport and transfer into a foreign group.

Keeper intervention during the introduction period helped to prevent serious confrontations between the elephants. This intervention was possible because the animals were well trained. This might also have been responsible for the absence of serious welfare problems in the study animals, and certainly made the handling of the animals during transport and introduction much easier. Transferring elephants in pairs may also be an option for reducing stress reactions, although here, of the two females transferred together, one still showed indications of stress.

Despite our findings that transfer and relocation of elephants for breeding may present only a weak stress to the animals concerned, it is necessary to consider whether this procedure is appropriate for promoting captive breeding of elephants. In the absence of information on the exact success rate of these breeding attempts, however, this is difficult to assess. Although other possibilities that would represent more natural circumstances — such as when bulls join female herds for breeding — should be promoted, the transfer of females between zoos will remain, at least in the near future, an important part of the conservation strategy of this species.

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