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A captive population in crisis: Testing hypotheses for reproductive failure in captive-born southern white rhinoceros females

Ronald R. Swaisgood^{a,*}, Dawn M. Dickman^{b,1}, Angela M. White^{a,2}

^aConservation and Research for Endangered Species, Zoological Society of San Diego, P.O. Box 120551, San Diego, CA 92112, USA

^bDepartment of Biology, University of California, San Diego, La Jolla, CA 92093, USA

ARTICLE INFO

Article history:

Received 1 September 2005

Received in revised form

10 November 2005

Accepted 18 November 2005

Available online 3 January 2006

Keywords:

Conservation breeding

Reproductive suppression

Conservation behavior

Reproductive behavior

Rhinoceros

ABSTRACT

The captive southern white rhinoceros (*Ceratotherium simum simum*) population is not self-sustaining. Many founders reproduced, but reproduction among captive-born (F_1) females has been extremely sluggish. Thus the conservation breeding program for this species faces a looming crisis. Using behavioral observations of a large captive population and a questionnaire survey circulated to facilities worldwide, several hypotheses for F_1 female reproductive failure were evaluated. Counter to predictions regarding behavioral deficiency in sociosexual behaviors, F_1 females were at least as proficient as F_0 females for all behavioral measures. Males also showed no sociosexual preferences for F_0 over F_1 females. Results indicate that most reproductive failure occurs post-copulation. The reigning root-cause hypothesis for F_1 female reproductive failure postulates that F_0 females are behaviorally dominant and suppress reproduction in F_1 females. However, no evidence for behavioral dominance was found and F_1 females housed with F_0 females were more likely to reproduce than those housed without F_0 females. Such social facilitation of reproduction is beneficial to F_1 female reproduction, but does not explain differential reproduction between F_1 and F_0 females. Because the design controlled for current conditions, these results point to development in captivity as the root cause of postcopulatory reproductive failure in F_1 females.

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1. Introduction

Captive breeding is a significant component of conservation to the extent that self-sustaining populations can be maintained as a genetic reservoir should they be needed for reintroduction or population supplementation (IUCN, 1998). Captive release programs have met with mixed success (Wolf et al., 1998; Fischer and Lindenmayer, 2000), yet can play a significant role in recovery of individual species (e.g., Frantzen

et al., 2001; O'Toole et al., 2002; Wanless et al., 2002; Britt et al., 2003; Green et al., 2005). The World Conservation Union (IUCN) recommends that captive breeding programs should be established before the in situ population becomes so precarious that removals will exacerbate its decline. However, many captive populations are not self-sustaining and some continue to rely on removals of animals from the wild. For captive populations in crisis behavioral research can play a critical role in identifying and solving problems with breeding

* Corresponding author: Tel.: +1 619 744 3372; fax: +1 619 744 3346.

E-mail address: rswaisgood@sandiegozoo.org (R.R. Swaisgood).

¹ Current affiliation for Dawn D. Dickman: Lewis & Clark Law School, Portland, OR 97219, USA.

² Current affiliation for Angela M. White: Environmental and Resource Sciences, University of Nevada Reno, Reno, NV 89512, USA.

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doi:10.1016/j.biocon.2005.11.015

and offspring survival (Kleiman, 1980; Lindburg and Fitch-Snyder, 1994; Wielebnowski, 1998; Swaisgood, 2004). Expression of appropriate social and reproductive behavior is one of the most common obstacles to conservation breeding, a problem that can be remedied by behavioral research (Lindburg and Fitch-Snyder, 1994; Wielebnowski, 1998; Swaisgood et al., 2000, 2003; Wanless et al., 2002; Fisher et al., 2003). Although informed changes in social management often successfully improve breeding success, few researchers have used a hypothesis-testing approach, which is necessary if the precise determinants of reproductive success and failure are to be understood.

We initiated a research program to address a crisis in conservation breeding for the southern white rhinoceros (*Ceratotherium simum simum*). White rhinoceros, formerly endangered, are now listed as conservation-dependent by the IUCN. More white rhinos have been exported from the wild than reside in captivity; thus captive breeding programs are failing. Global captive white rhino annual growth rate is projected at negative 3.5%, whereas growth rates in wild populations are 6–10% (Emslie and Brooks, 1999). The IUCN still supports captive breeding of white rhino as a safety net in case the political and social instability in the range countries reverses current population trends, as dramatically illustrated in other species such as black (*Diceros bicornis*) and Northern white (*Ceratotherium simum cottoni*) rhino (Emslie and Brooks, 1999). Many of the founding captive population of southern white rhino, given appropriate husbandry and management, reproduced well. Crucial to that success was the housing of rhinos in larger enclosures containing at least one male and several females (Rawlings, 1979; Lindemann, 1982; Fouraker and Wagener, 1996), an environment that would be more consistent with conditions in the wild, where males have access to several females and breed polygynously (Owen-Smith, 1975). However, reproduction among captive-born (F_1) females has been extremely sluggish (Emslie and Brooks, 1999; AZA, 2004), with as few as 8% reproducing in some populations (Schwartzberger et al., 1999). Because males continued to sire offspring with wild-caught (F_0) females, the problem lies with the F_1 females. This situation is destined to deteriorate further as the F_0 females that formerly drove population growth begin to die off. Thus there is an urgent need to solve this conservation breeding problem soon or face the dilemma of further captures of wild rhinos to support the captive population.

Significant headway has been made in trying to determine what factors cause reproductive failure in F_1 females. For example, a number of investigations have detailed the pattern of reproductive hormones across the reproductive cycle (Schwartzberger et al., 1998; Patton et al., 1999; Brown et al., 2001; Carlstead and Brown, 2005). Although anomalies in the reproductive cycle have been found, they are no more common in captive-born than wild-caught females, and so do not explain why more captive-born females fail to reproduce (Schwartzberger et al., 1998; Patton et al., 1999). Endocrine data also indicate that age does not affect cyclicity (Brown et al., 2001). Why do white rhino females breed well when brought into captivity from the wild, but produce F_1 female offspring that fail to reproduce? There are plenty of examples of reproductive problems in conservation breeding

programs, but we are not aware of any where the primary problem is limited to the F_1 generation. The case of the white rhinoceros is one of the great unsolved mysteries of animal reproduction in zoological institutions, a problem which has remained intractable despite considerable previous scientific effort.

Several hypotheses have been advanced to explain reproductive failure in F_1 females. Some of the hypotheses are specific to the immediate causal mechanism (e.g., abnormal endocrine cycles discussed above), while others postulate root causes. By far the root-cause hypothesis most strongly advocated by rhino managers and scientists is reproductive suppression of F_1 females by the older F_0 females sharing the enclosure (Anonymous, 2001; Carlstead and Brown, 2005); however, there are no data to support this contention. Reproductive suppression, in which dominant individuals suppress reproduction in subordinates at behavioral and/or physiological levels, has been observed in several mammalian species. These species, however, tend to be highly social with well developed dominance relationships (Abbott, 1987; Faulkes et al., 1990; Creel et al., 1992; Solomon and French, 1996; Creel et al., 1997), a social system quite divergent from the relatively fluid social system of white rhinoceros in nature (Swaisgood, unpublished data, Owen-Smith, 1975). Moreover, these field studies revealed no evidence of reproductive suppression or dominance. Nonetheless, the prevalence of reproductive failure among F_1 females raises the possibility that captivity may somehow promote stress and reproductive suppression, perhaps as a result of social density (reviews in: Hoffer and East, 1998; Wielebnowski, 1998; Morgan and Tromborg, in press). Indeed the cheetah (*Acinonyx jubatus*) does not possess a social system typically seen in species with reproductive suppression, yet ovarian cyclicity is suppressed by the presence of female conspecifics in captivity (Wielebnowski et al., 2002).

Here we test several predictions of the reproductive suppression hypothesis in white rhinoceros. We also attempt to pinpoint where in the chain of events necessary for successful reproduction the breakdown takes place, by comparing several measures of reproductive proficiency in F_1 and F_0 females.

2. Methods

2.1. Observational study

Behavioral observations were made on 6 F_0 adult females (wild-born) and 5 F_1 (captive-born) adult southern white rhinoceros females residing in a 90-acre enclosure at the San Diego Zoo's Wild Animal Park (SDZWAP). For details see Patton et al. (1999). There was only one adult male present at a time, but males were exchanged midway through the study (both were wild-born). There were between 2 and 4 subadults present. Adults were females >5 years of age or females displaying regular reproductive endocrine cycles (Patton et al., 1999). Group composition changed during the 4-year study, consisting of 11–14 individuals at any given time.

Daily observations were made during active periods (unpublished data), the first and last 3 h of daylight. A total of 3827 h of data was collected using 1-h focal-animal

samples and instantaneous scan samples (Martin and Bateson, 1993). Behaviors are defined in Table 1. The unit of analysis was each female–female dyad for the dominance study. For the sociosexual behavior study we used each male–female dyad in each of three phases of the estrous cycle: pre-estrus (2–6 days before ovulation), peak estrus (day before and day of ovulation) and nonestrus (more than 2 days after ovulation). Ovulation was inferred from observed mounting/mating, evidence of mounting (e.g., hoofmarks on back, semen), and, when available, endocrine indicators of ovulation (Patton et al., 1999). In this previous study, evidence of mating always coincided with endocrine indicators of ovulation, thus mating is a reliable indicator of the fertile period. We used repeated-measures ANOVA with one within- (estrous phase) and one between-subject (female origin) factor. Cycle length varied from 28–79 days (see also Patton et al., 1999). Using estrous phase as a blocking variable ensured that differences between F_1 and F_0 females cannot be attributed to amount of time spent in different phases of estrus, which effectively removes any confound that age may have on reproductive activity (F_1 females were inevitably younger than F_0 females).

2.2. Questionnaire study

We circulated a questionnaire to 90 facilities worldwide housing F_1 females; of these, 40 responded and 21 were included in the analysis. Females under 9 years of age were excluded from the analysis, thus giving included females ample time to display normal reproductive behavior as adults (puberty onset varies from 2.5 to 4.5 years of age and age of first reproduction ranges from 5.6–8 years of age (Owen-Smith, 1988; Patton et al., 1999)). Other females were excluded because they had been at the facility <4 years or had access to a male <9 months/year, yielding a sample size of 28 F_1 and 27 F_0 females. N varies in questionnaire data because respondents

Table 2 – Partial list of questions for questionnaire study

Requested information
Group age-sex composition, current and historical
Enclosure size
Management of male–female contact (time housed separately)
Animal origin (wild- or captive-born)
Time spent at current facility
Dominance status
Frequency of behavioral estrus
Frequency of male courtship of female
Male courtship behaviors observed with female
Record of copulations
Record of pregnancies and births

did not provide information for all questions for all females. Table 2 provides an overview of the data collected.

3. Results

3.1. Comparing F_1 and F_0 females' reproductive proficiency

3.1.1. Hypothesis 1: Do F_0 females display more proficient sociosexual behavior towards males than do F_1 females?

Our findings from SDZWAP do not corroborate this hypothesis. F_1 and F_0 females did not differ with regard to the number of affiliative acts (Fig. 1b; $F_{2,21} = 0.05$, $p > 0.50$) or aggressive acts (Fig. 1d; $F_{2,21} = 0.7$, $p > 0.50$) that they directed towards males (including mutual acts). Table 3 further indicates that none of the 12 individual behaviors contributing to this analysis were consistent with this hypothesis, despite using a very liberal statistical approach wherein we did not control for familywise error rate. Indeed, F_1 females were significantly more likely to approach males and significantly less likely to exhibit the aggressive snarl display toward males than were

Table 1 – Definitions of behaviors recorded for SDZWAP study

Behavior	Definition
Approach	Moves to within 1 body length of another rhino
Follow	Follows another animal for >5 body lengths
Hic vocalization	Repetitive breathy vocalization
Greet	Nasonasal contact or near contact
Horn wrestle	Slow lateral fencing movements with horns pressed together
Contact	Any other non-aggressive contact with another rhino
Proximity	Percentage time within 3 body lengths of another rhino
Deter	Arrests the approach of another rhino by turning to face, head tossing, mock charging, etc.
Displace	Other rhino moves away upon the approach or behavior of subject rhino
Charge	Rapid aggressive approach of another individual
Snarl display	A loud rasping roar with head thrust forward, ears laid back, and mouth open
Chase	Vigorous pursuit of another individual for >5 body lengths
Horn	Aggressive contact of horn to body; attempt to gore
Horn clash	Aggressive clash of horns with another rhino
Anogenital investigation	Sniffs anogenital area of another rhino
Chin rest	Male rests his head on female's hind quarters
Mount	Male on hind legs, mounted female from behind with penis in the vicinity of female's genitalia
Copulation	Intromission: male inserts penis in female's vagina
Ejaculation	Episodic quivering in male's hind legs during copulation
Hold	Female remains standing for >5 s when male attempts to chin rest or mount

N.B. Many definitions sensu Owen-Smith (1975).

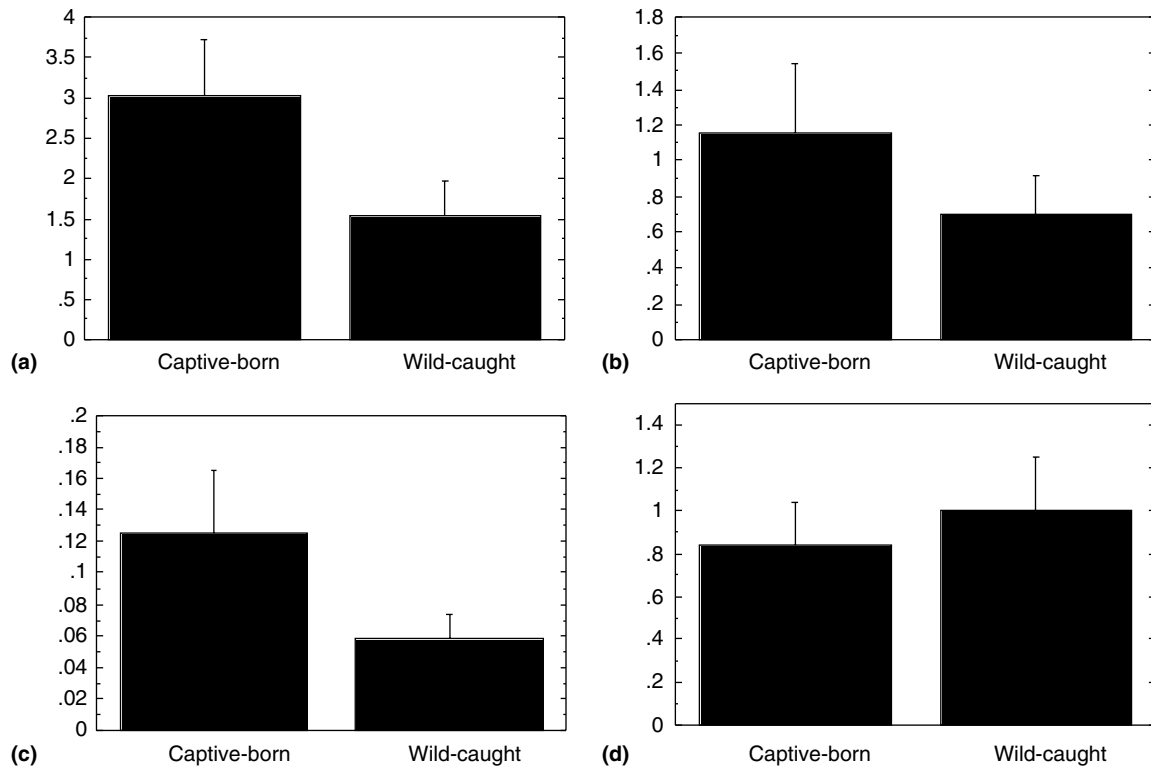


Fig. 1 – The rate of social behaviors between males and females as a function of female origin. Data are the sum of all affiliative or aggressive behaviors and presented as mean rate per hour + standard error. (a) Total number of affiliative acts that males directed toward females; (b) total number of affiliative acts that females directed toward males; (c) total number of aggressive acts that males directed toward females; (d) total number of aggressive acts that females directed toward males.

F₀ females, results directly counter to predictions from this hypothesis. Table 3 also indicates that F₁ and F₀ females are equally receptive to male courtship advances during peak estrus, as indicated by their willingness to hold still while the male attempts to chin rest.

The questionnaire data also fail to support this hypothesis. F₁ females were more likely to exhibit behavioral signs of estrus (95.7%) than F₀ females (57.7%; $\chi^2 = 9.5$, $n = 49$, $p = 0.02$).

3.1.2. Hypothesis 2: Do males exhibit sociosexual preferences for F₀ over F₁ females?

SDZWAP data do not support this hypothesis. In fact, males directed affiliative behavior toward F₁ more often than toward F₀ females, although this effect was not significant (Fig. 1a; $F_{2,21} = 3.1$, $p < 0.10$). Male aggression was exceedingly rare, but males also directed more aggressive acts toward F₁ than F₀ females, although this effect did not even approach significance (Fig. 1c; $F_{2,21} = 1.9$, $p > 0.10$). Taken together, these findings indicate that the somewhat higher aggression rate was a byproduct of higher interaction rates with F₁ females, not an aggressive disposition toward them. Table 3 shows just how rare and biologically insignificant male aggression appears to be. It also shows a strong trend for males to display more affiliative behavior toward F₁ females, following F₁ females significantly more and approaching them twice as often as they did F₀ females (albeit nonsignificantly). Finally, during peak estrus, males were equally likely to direct courtship behaviors toward F₁ and F₀ females (Table 3).

Likewise our questionnaire data are inconsistent with this hypothesis (Table 4). Male sociosexual/courtship behaviors were equally likely to be directed toward F₁ and F₀ females.

3.1.3. Hypothesis 3: Is copulatory behavior compromised in F₁ females?

SDZWAP data do not support this hypothesis. Comparisons of means presented in Table 3 suggest no meaningful differences in courtship behaviors between F₁ and F₀ females. Our SDZWAP data on copulatory behavior is more limited because most mounting and copulation behavior did not occur during observation periods. We observed seven copulations for two F₁ females and two copulations in one F₀ female. For the two F₁'s the average copulation lasted 16.8 and 14.1 min, compared to 14.1 min for the F₀ female. The form of copulation was also the same for the F₁'s and F₀'s, with both male and female remaining relatively stationary until dismounting. Females were equally likely to “hold” while the male attempted to mount: 90.7% and 92.7% for the F₁'s and 95.8% for the F₀. The number of ejaculations per copulation was also comparable among the females: 8.4 and 7.6 for the F₁'s and 7.6 for the F₀. In sum, there is no compelling evidence from these limited data that F₁ and F₀ females differ with regard to reproductive behavior potentially related to fertilization success.

Our questionnaire data indicate that F₀ females may be more likely to copulate than F₁ females, although this effect is nonsignificant ($\chi^2 = 3.3$, $p = 0.07$) and does not take into ac-

Table 3 – Comparison of male–female sociosexual behavior for captive-born (F_1) and wild-caught (F_0) females using SDZWAP data

Behavior	Captive-born	Wild-caught	p-Value ^a	Hypothesis ^b
<i>Female affiliative</i>				
-F follow M	0.01 ± 0.002	0.02 ± 0.001	NA	NA
-F approach M	0.56 ± 0.09	0.25 ± 0.06	$p < 0.025$	I*
<i>Male affiliative</i>				
M follow F	0.56 ± 0.12	0.22 ± 0.06	$p < 0.05$	I*
M approach F	1.85 ± 0.37	0.87 ± 0.24	$p < 0.10$	I
M hic vocalization	0.40 ± 0.15	0.26 ± 0.12	$p > 0.75$	N
<i>Mutual affiliative</i>				
-Greet	0.08 ± 0.03	0.08 ± 0.03	$p > 0.50$	N
-Contact	0.25 ± 0.09	0.29 ± 0.14	$p > 0.25$	N
-Horn wrestle	0.20 ± 0.15	0.06 ± 0.02	$p > 0.25$	I
-Proximity (% time)	8.1 ± 1.1	5.8 ± 1.2	$p > 0.75$	N
<i>Female aggressive</i>				
-F deter M	0.17 ± 0.04	0.17 ± 0.05	$p > 0.50$	N
-F displace M	0.47 ± 0.13	0.54 ± 0.17	$p > 0.50$	N
-F charge M	0.05 ± 0.04	0.06 ± 0.01	NA	NA
-F snarl display M	0.07 ± 0.03	0.21 ± 0.04	$p < 0.001$	I*
-F chase M	0.0004 ± 0.0003	0.001 ± 0.0004	NA	NA
-F horn M	0.005 ± 0.005	0.0003 ± 0.0003	NA	NA
<i>Male aggressive</i>				
-M deter F	0.003 ± 0.01	0.001 ± 0.0004	NA	NA
-M displace F	0.08 ± 0.07	0.03 ± 0.01	NA	NA
-M charge F	0.02 ± 0.02	0.01 ± 0.002	NA	NA
-M snarl display F	0.003 ± 0.002	0.01 ± 0.002	NA	NA
-M chase F	0.002 ± 0.03	0.001 ± 0.001	NA	NA
-M horn F	0.004 ± 0.003	0.001 ± 0.0004	NA	NA
<i>Mutual aggressive</i>				
-Horn clash	0.01 ± 0.02	0.02 ± 0.01	NA	NA
<i>Courtship^c</i>				
-M anoinvestigate F	1.67 ± 0.54	1.81 ± 0.46	NA	N
-M chin rest F	1.44 ± 0.29	2.27 ± 0.8	NA	N
-F holds for chin rest (%)	79.58 ± 4.83	82.75 ± 0.55	NA	N

Source: 11 females and 2 males residing at SDZWAP.

N.B. These analyses on multiple dependent variables are not independent and are included here to illustrate the behavioral details supporting the analysis of the four combined behavior patterns in Fig. 1. While not statistically valid per sé, this liberal approach maximizes the chances that at least some behaviors will be consistent with the predictions from the hypothesis that F_1 females are compromised in their socio-sexual behavior. However, these data show that none of the behavior patterns studied are consistent with this hypothesis.

a Behaviors that occurred at a rate of less than 0.1/h for either captive-born or wild-caught females were not analyzed (NA) because of their dubious biological significance. Behaviors only observed during peak estrus (i.e., courtship) were also not analyzed. These criteria also excluded all dependent variables that did not meet the distributional assumptions for ANOVA. p-Values reported are from ANOVA ($F_{2,21}$).

b When behavior by or toward F_1 females occurred at twice the rate as for F_0 females, or vice-versa, we labeled the results from this behavior as consistent (C) or inconsistent (I) with the hypothesis and predictions stated in Section 3.

c Behavioral values for courtship behaviors are based on data collected during peak estrus only. These behaviors were rarely or never observed during other phases of the estrous cycle.

* An asterisk (*) indicates whether the difference is statistically significant. If this criterion was not met, we concluded that differences between F_1 and F_0 females was not likely to be biologically significant and accepted the null hypothesis (N) indicating that F_1 and F_0 females did not differ with regard to this behavioral variable.

count that F_0 females had more years of opportunity to copulate. Because copulation is rarely observed unless observers are present for many hours/day, records on copulations may not be accurate.

3.1.4. Hypothesis 4: Are F_1 females less likely to conceive or maintain pregnancy than F_0 females?

Our SDZWAP data provide support for this hypothesis. Keeper and researcher records confirm that 5/5 F_1 females copulated during the course of the study, compared with 4/6 F_0 females.

Only one of the F_1 females produced an offspring, whereas three of the F_0 females did, suggesting that reproductive failure can be attributed to the post-copulation period. Our questionnaire data provide even stronger support for this hypothesis. Of the 18 F_1 females that were known to copulate only 39% produced offspring, whereas 79% of the 34 F_0 females that copulated produced offspring ($\chi^2 = 6.8$, $p = 0.009$). To control for years of opportunity, we also analyzed data only for births that occurred before the age of 15 among females that reached that age, and found further support that F_1

Table 4 – Comparison of male–female sociosexual for captive-born (F_1) and wild-caught (F_0) females using questionnaire data

Behavior	Captive-born	Wild-caught	Test statistic	Hypothesis ^b
Anogenital investigation	69.0%	55.5%	$p > 0.25$	N
Chin rest	80.0%	60.0%	$p > 0.10$	N
Hic vocalization	59.5%	35.5%	$p > 0.25$	N
Mount	70.0%	58.0%	$p > 0.50$	N

Source: questionnaire data from 28 F_1 and 27 F_0 females residing in 21 facilities. Data presented are percentages of individuals displaying behavior. p -Values reported are from χ^2 tests (degrees of freedom = 1; $N = 49$).

^b N = null hypothesis accepted; see Table 3.

females are reproductively compromised compared to F_0 females ($F_1 = 6/15 = 34\%$; $F_0 = 21/27 = 78\%$; $\chi^2 = 8.9$, $p = 0.003$).

3.2. Reproductive suppression: evaluating whether female dominance influences reproductive failure

3.2.1. Hypothesis 1: Is there evidence for behavioral dominance between F_1 and F_0 females?

Our SDZWAP data on nonpartner females provide some support for dominance in white rhinos. For most of the aggressive behaviors, too few females ever displayed them toward other females; thus no analysis was possible. Table 5 includes the two aggressive behaviors observed enough to merit analysis, but also includes “approach” to determine if other aspects of social interaction may be asymmetrical between F_1 and F_0 females. This table shows that approach rates were similar, but both F_0 and F_1 females displaced and snarled at F_1 females more than they did to F_0 females (at more than twice the rate, but statistical analysis was not possible). This finding is consistent with the hypothesis that F_0 females are dominant. However, aggression is so rare that it is perhaps biologically insignificant.

If dominance exists in white rhino female society, it is most likely to be expressed between individuals in long-term relationships. All partnerships consisted of one F_0 and one ($n = 4$) or two ($n = 1$) F_1 females; thus, partner data are not available for F_1 – F_1 or F_0 – F_0 interactions. Partnerships were easily identified, both subjectively and by analyses showing that some females were more likely to be in proximity with each other. Most aggressive behaviors were never seen between partner females and the remaining were so rare that no analysis was possible. For example, a total of 6 snarl displays were seen between partners. Mann–Whitney U -tests revealed no asymmetry in the rate of approaches by F_1 to F_0 (1.23 ± 0.23) versus F_0 to F_1 (1.36 ± 0.20) partners ($U = 0.3$, $n = 6, 5$, $p = 0.75$).

Questionnaire data indicate that animal caretakers perceive relationships between female white rhinos that indicate dominance, though we do not know what behaviors they use to make this judgment. Nonetheless, these data show that F_0 females (37.5%) are not more likely to be dominant than F_1 females (38.5%; $\chi^2 = 0.003$, $n = 37$, $p = 0.95$).

3.2.2. Hypothesis 2: Do F_0 females suppress reproductive behavior or reproduction among F_1 females?

As indicated above, SDZWAP data provide no evidence that F_0 females suppress reproductive behavior of F_1 females. Our

questionnaire data likewise provide no support for this hypothesis. F_1 female behavioral estrus was not affected by the presence of F_0 females: 12/12 housed with F_0 females exhibited behavioral estrus, compared with 10/11 for those housed without F_0 females (Fisher’s exact test: $p > 0.20$). Males were also equally likely to court F_1 females in the presence (12/12) or absence (9/11) of F_0 females ($p > 0.50$). Most importantly, F_1 females were significantly more likely to reproduce in the presence (6/13) than the absence (1/14) of F_0 females (Fisher’s exact test: $p < 0.05$).

A sub-hypothesis is that mothers suppress reproduction in their daughters if housed together as adults. This prediction also is not supported by our data. F_1 females were equally likely to exhibit behavioral estrus while living with (6/7) or without their mother (17/17; Fisher’s exact test: $p > 0.20$) and males were equally likely to court them (6/7 and 15/16, respectively; Fisher’s exact test: $p > 0.50$). In addition, 4/8 F_1 females gave birth while living with their mothers, whereas 3/20 living without their mothers did so (Fisher’s exact test: $p < 0.001$), results directly counter to the maternal suppression hypothesis.

4. Discussion

These two studies suggest that F_1 females have normal behavioral estrus and display normal social and sexual behaviors toward males. When compared with F_0 females that have reproduced well, F_1 females, if anything, show more proficient behavior. Similarly, reproductive failure in F_1 females cannot be attributed to lack of male interest, for males showed more affiliative social interest in F_1 than F_0 females (marginally nonsignificant) and were equally likely to court the two classes of female. SDZWAP data suggest no copulatory deficiencies in F_1 females, but the questionnaire data may indicate that F_1 females are less likely to copulate than F_0 females (marginally nonsignificant), but F_1 females had fewer years of opportunity to copulate. Whether F_1 females suffer from copulatory deficiencies must await further analysis. However, our data clearly show that F_1 females that have copulated are still less likely to produce offspring than are F_0 females, even when we controlled for years of reproductive opportunity. Thus, our most important finding is that F_1 females must experience reproductive problems post-copulation, failing to conceive or maintain the pregnancy. These studies also show how testing a series of hypotheses can help pinpoint where in the chain of events reproductive failure occurs.

Table 5 – Directionality of social behaviors between non-partner females that may indicate dominance relationships between F₁ and F₀ females

Behavior	A	R	X ± SE	A	R	X ± SE	Hypothesis
Approach	F ₁	F ₁	0.21 ± 0.12	F ₁	F ₀	0.12 ± 0.05	N
Approach	F ₀	F ₀	0.07 ± 0.01	F ₀	F ₁	0.11 ± 0.02	N
Displace	F ₁	F ₁	0.007 ± 0.002	F ₁	F ₀	0.002 ± 0.001	C
Displace	F ₀	F ₀	0.033 ± 0.014	F ₀	F ₁	0.004 ± 0.002	C
Snarl	F ₁	F ₁	0.002 ± 0.001	F ₁	F ₀	0.0002 ± 0.0002	C
Snarl	F ₀	F ₀	0.0015 ± 0.0006	F ₀	F ₁	0.007 ± 0.002	C

Source: 6 F₀ and 5 F₁ females residing at SDZWAP. A: Actor; R: Recipient. Small sample size for within-subjects tests precludes statistical analysis, as significance cannot be attained at the $p = 0.05$ level. Data are averaged for each female towards all non-partner F₁ females and all non-partner F₀ females. The hypothesis predicts that F₀ females will show signs of behavioral dominance over F₁ females. Each line tests a specific hypothesis. For example, line 1 poses the question, “Do F₁ females approach other F₁ females more than they approach F₀ females?” For codes in hypothesis column see Table 3. Because all female–female behavioral measures for these hypotheses were so infrequent we relaxed the criterion that the rate must be >0.1/h.

We also tested the reigning root-cause hypothesis for reproductive failure in F₁ females, namely, that dominant F₀ females suppress reproduction in subordinate F₁ females behaviorally or physiologically. This hypothesis presupposes that behavioral dominance exists. Nearly all definitions of dominance require an asymmetry in the behavioral relationship, especially with regard to aggressive behavior and access to resources (review in Barrows, 2001). Mikulica (1991) found evidence for dominance in a 220-h study of captive southern white rhinoceros. However, we could find only limited evidence consistent with dominance in our hundreds of hours of observation at SDZWAP. Although F₁ females were more often the target of some aggressive behaviors than were F₀ females, aggression between females (whether partners or nonpartners) was exceedingly rare and we could not analyze it statistically. Analysis of approach rates indicated no behavioral asymmetry, as might be expected if relationships were unequal. We also found no common female–female behaviors that could be classified as “submissive” or “appeasement” signals, behaviors that characterize most dominance relationships. The differences between our findings and those of Mikulica (1991) may be attributed to methodological differences or the fact that social density was much lower in the larger SDZWAP enclosure. Regardless, despite the lack of female–female aggression in our study, F₁ females still experienced reproductive problems. Our questionnaire data also revealed no difference in dominance status between F₀ and F₁ females. In a recent study of wild rhino, we have also found no evidence for female–female dominance or reproductive suppression (Swaisgood, unpublished data). Dominance could be an artifact of captivity, but one would not expect it to be mediated subtly—especially in a marginally social species—since subtle, as opposed to conspicuous, signals imply signaler–receiver coevolution where the receiver also has clear benefits from its response to the signal (Krebs and Dawkins, 1984). Even in highly social primates or canids, where subordinates often have little option but to acquiesce to dominant individuals, dominance signals are not so subtle. Given that social living is not obligate in white rhinos, why would they forgo reproduction without significant aggression to back up subtle signals? We suspect that white rhino females do not possess a dominance

system well-developed enough to suppress reproduction, even in captivity.

More importantly, we were unable to find any evidence that the presence of F₀ females or, more specifically, the mothers, suppressed reproductive behavior, or reproduction in F₁ females. In fact, the opposite was true: F₁ females living with F₀ females or F₀ mothers were significantly more likely to reproduce. These data suggest that the presence of other adult females enhances reproduction. Indeed, we have preliminary data (unpublished) that supports this hypothesis, showing that females reproduce better with increasing group size (see also Rawlings, 1979; Lindemann, 1982; Fouraker and Wagener, 1996); however, the effects are equivalent for F₁ and F₀ females, promoting reproduction in both, so these husbandry factors cannot explain differential reproduction between F₁ and F₀ females. Nonetheless, it is clear that F₁ females should be kept in enclosures with other females, regardless of their origin, to maximize reproduction, if not equalizing it with F₀ females. Future research may indeed expose some cases of reproductive suppression, but our data strongly indicate that it is not a major cause of reproductive failure in F₁ females, and we suggest that attention should be focused elsewhere.

5. Conclusions and future directions

The research reported here dramatically narrows the scope in the search for causes of F₁ female white rhino reproductive failure and points the direction to new studies that may ultimately solve the mystery. We point out that multiple-institution research is the only real chance we have of determining root causes, that is, aspects of the captive environment that lead to reproductive failure. Other intensive research on one or a few captive populations, while useful and necessary, will only be able to discern the symptoms (e.g., reproductive pathologies).

A strength of our studies was that the experimental designs ensured that F₁ and F₀ females were kept at the same institutions and exposed to the same social, environmental and management circumstances; yet reproduction in F₁ females was significantly less than in F₀ females. Because all factors were held constant at the time of our studies, our results point strongly toward a negative impact of captivity

during the development of F₁ females; that is, the circumstances of our subjects differed only during their early years, with some females developing in the wild and some in captivity. This strongly implicates the captive environment during development as the ultimate causal factor.

Future research should focus on developmental effects on reproduction, as well as comparative studies on development and reproduction in the wild. Studies comparing behavior of wild and captive animals can be extremely useful, both for developing optimal captive management (Kleiman, 1980) and guiding reintroduction programs (Mathews et al., 2005). We have recently completed a field study of white rhino (unpublished) and will examine data for insights into the F₁ reproduction problem. Also planned are follow up studies comparing white rhino development and reproduction in populations that vary from highly captive to semi-wild in the source country, South Africa. We urge the scientific community to address the F₁ reproduction problem quickly or we will face the prospect of importing more rhino from the wild, which has dubious conservation and ethical merit.

Acknowledgements

We are grateful to Randy Rieches for supporting the project and to several volunteers who assisted with data collection, most notably J.P. Montagne, Tom Janes, Shannon Chamberlain, and Patty Gaffney. Financial support was provided by the Zoological Society of San Diego.

REFERENCES

- Abbott, D.H., 1987. Behaviourally mediated suppression of reproduction in female primates. *Journal of Zoology*, London 213, 455–470.
- Anonymous, 2001. In: Proceedings of the Rhino keepers' Workshop. Zoological Society of San Diego, San Diego.
- AZA, 2004. AZA Rhino Research Advisory Group: Five-year Research Masterplan. American Zoo and Aquarium Association.
- Barrows, E.M., 2001. *Animal Behavior Desk Reference: A Dictionary of Animal Behavior, Ecology, and Evolution*. CRC Press, Boca Raton, FL.
- Britt, A., Welch, C., Katz, A., 2003. Can small, isolated primate populations be effectively reinforced through the release of individuals from a captive population? *Biological Conservation* 115, 319–327.
- Brown, J.L., Bellem, A.C., Fouraker, M., Wildt, D.E., Roth, T.L., 2001. Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in North America by noninvasive endocrine monitoring. *Zoo Biology* 20, 463–486.
- Carlstead, K., Brown, J.H., 2005. Relationships between patterns of fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. *Zoo Biology* 24, 215–232.
- Creel, S., Creel, N., Wildt, D.E., Monfort, S.L., 1992. Behavioural and endocrine mechanisms of reproductive suppression in dwarf mongooses. *Animal Behaviour* 43, 231–245.
- Creel, S., Creel, N., Mills, M.G.L., Monfort, S.L., 1997. Rank and reproduction in cooperatively breeding African wild dogs: behavioral and endocrine correlates. *Behavioral Ecology* 8, 298–306.
- Emslie, R., Brooks, M., 1999. African Rhino Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland and Cambridge, UK.
- Faulkes, C.G., Abbott, D.H., Jarvis, J.U.M., 1990. Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats, *Heterocephalus glaber*. *Journal of Reproduction and Fertility* 88, 559–568.
- Fischer, J., Lindenmayer, D.B., 2000. An assessment of the published results of animal relocations. *Biological Conservation* 96, 1–11.
- Fisher, H.S., Swaisgood, R.R., Fitch-Snyder, H., 2003. Odor familiarity and female preferences for males in a threatened primate, the pygmy loris, *Nycticebus pygmaeus*: applications for genetic management of small populations. *Naturwissenschaften* 90, 509–512.
- Fouraker, M., Wagener, T., 1996. AZA Rhinoceros Husbandry Resource Manual. Fort Worth Zoological Park, Fort Worth, TX.
- Frantzen, M.A.J., Ferguson, J.W.H., de Villiers, M.S., 2001. The conservation role of captive African wild dogs (*Lycan pictus*). *Biological Conservation* 100, 253–260.
- Green, A.J., Fuentes, C., Figuerola, J., Viedma, C., Ramón, N., 2005. Survival of marbled teal (*Marmaronetta angustirostris*) released back into the wild. *Biological Conservation* 121, 595–601.
- Hofer, H., East, M.L., 1998. Biological conservation and stress. *Advances in the Study of Behavior* 27, 405–525.
- IUCN, 1998. IUCN guidelines for re-introductions. IUCN/SSC Re-introduction Specialist Group, Gland, Switzerland.
- Kleiman, D.G., 1980. The sociobiology of captive propagation in mammals. In: Soule, M.E., Wilcox, B.A. (Eds.), *Conservation Biology: An Evolutionary–Ecological Perspective*. Sinauer Associates, Sunderland, MA, pp. 243–262.
- Krebs, J.R., Dawkins, R., 1984. Animal signals: mind-reading and manipulation. In: Krebs, J.R., Davies, N.B. (Eds.), *Behavioural Ecology An Evolutionary Approach*, second ed. Sinauer, Sunderland, MA, pp. 380–402.
- Lindburg, D.G., Fitch-Snyder, H., 1994. Use of behavior to evaluate reproductive problems in captive mammals. *Zoo Biology* 13, 433–445.
- Lindemann, H., 1982. African Rhinoceroses in Captivity. Ph.D. thesis, University of Copenhagen, Copenhagen, Denmark.
- Martin, P., Bateson, P., 1993. *Measuring Behaviour*. Cambridge University Press, Cambridge.
- Mathews, F., Orros, M., McLaren, G., Gelling, M., Foster, R., 2005. Keeping fit on the ark: assessing the suitability of captive-bred animals for release. *Biological Conservation* 121, 569–577.
- Mikulica, V., 1991. Social behaviour in two captive groups of white rhinoceros (*Ceratotherium simum simum* and *Ceratotherium simum cottoni*). *Der Zoologische Garten* 61, 365–385.
- Morgan, K.N., Tromborg, C.T. Sources of stress in captivity. In: Swaisgood, R.R. (Eds.), *Special Issue: Animal Behavior, Conservation and Enrichment*. Applied Animal Behavior Science, in press.
- O'Toole, L., Fielding, A.H., Haworth, P.F., 2002. Re-introduction of the golden eagle into the Republic of Ireland. *Biological Conservation* 103, 303–312.
- Owen-Smith, N., 1975. The social ethology of the white rhinoceros *Ceratotherium simum* (Burchell 1817). *Zeitschrift für Tierpsychologie* 38, 337–384.
- Owen-Smith, N., 1988. Megaherbivores: the Influence of Very Large Body Size on Ecology. Cambridge University Press, Cambridge.
- Patton, M., Swaisgood, R., Czekala, N., White, A., Fetter, G., Montagne, J., Lance, V., 1999. Reproductive cycle length in southern white rhinoceros (*Ceratotherium simum simum*) as determined by fecal pregnane analysis and behavioral observations. *Zoo Biology* 18, 111–127.

- Rawlings, C.G.C., 1979. The breeding of white rhinos in captivity – a comparative survey. *Der Zoologische Garten* 49, 1–7.
- Schwartzberger, F., Walzer, C., Tomasova, K., Vahala, J., Meister, J., Goodrowe, K., Zima, J., Straub, G., Lynch, M., 1998. Faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in the white rhinoceros (*Ceratotherium simum*). *Animal Reproduction Science* 53, 173–190.
- Schwartzberger, F., Walzer, C., Tomasova, K., Zima, J., Göritz, F., Hermes, R., Hildebrand, T.B., 1999. Can the problems associated with the low reproductive rate in captive white rhinoceros (*Ceratotherium simum*) be solved in the next 5 years? *Verh. ber. Erkr. Zootiere* 39, 283–289.
- Solomon, N.G., French, J.A., 1996. *Cooperative Breeding in Mammals*. Cambridge University Press, Cambridge.
- Swaigood, R.R., 2004. Captive breeding. In: Bekoff, M. (Ed.), *Encyclopedia of Animal Behavior*. Greenwood Press, pp. 883–888.
- Swaigood, R.R., Lindburg, D.G., Zhou, X., Owen, M.A., 2000. The effects of sex, reproductive condition and context on discrimination of conspecific odours by giant pandas. *Animal Behaviour* 60, 227–237.
- Swaigood, R.R., Zhou, X., Zhang, G., Lindburg, D.G., Zhang, H., 2003. Application of behavioral knowledge to giant panda conservation. *International Journal of Comparative Psychology* 16, 65–84.
- Wanless, R.M., Cunningham, J., Hockey, P.A.R., Wanless, J., White, R.W., Wiseman, R., 2002. The success of a soft-release reintroduction of the flightless Aldabra rail (*Dryolimnas [cuvieri] aldabranus*) on Aldabra Atoll, Seychelles. *Biological Conservation* 107, 203–210.
- Wielebnowski, N., 1998. Contributions of behavioral studies to captive management and breeding of rare and endangered mammals. In: Caro, T. (Ed.), *Behavioral Ecology and Conservation Biology*. Oxford University Press, Oxford, pp. 130–162.
- Wielebnowski, N., Ziegler, K., Wildt, D.E., Lukas, J., Brown, J.L., 2002. Impact of social management on reproductive, adrenal and behavioural activity in the cheetah (*Acinonyx jubatus*). *Animal Conservation* 5, 291–301.
- Wolf, C.M., Garland, T., Griffith, B., 1998. Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts. *Biological Conservation* 86, 243–255.