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Social and Sexual Behavior of Two Newly Formed Pairs of Mexican Gray Wolf in Captivity

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Abstract: The social and maintenance behaviors of two pairs of Mexican gray wolf in captivity were studied. Wolves belonged to Zacango (ZZ) and León Zoo (LZ). For the ZZ pair significant differences were observed ($p<0.01$) for the social behaviors, whereas no differences were found for neutral or affiliative behaviors. The LZ pair showed no differences in social behavior before the breeding season, but after this significant differences were observed for playing and sexual activity ($p<0.001$). Wolves showed a preference ($p<0.0001$) to perform their activities on a reduced place far away from the human transit, especially those related with the courtship behavior. For the ZZ pair a total number of 389 mounting observations were registered during 11 consecutive days, most of them with pelvic movements but only two culminating with copulation (0.6%). The LZ pair showed a reproductive activity of 180 mountings, three of them culminating with copula (1.6%); none of the pairs showed reproductive success. Reproductive success depends on both physiological and behavioral compatibility, but some other causes may be involved in the reproductive failure of the ZZ pair, among them the stress of restraining for the cytology studies and the housing conditions like the presence of large predators as closer neighbors to the wolves.

Key words: Sexual behavior, social behavior, *Canis lupus baileyi*, Mexican gray wolf, reproductive season

Introduction

Canine social and reproductive behavior have been documented over the years (Mech, 1970; Servín, 1984, 1991; Packard *et al*., 1983; Asa *et al*., 1986, 1987; Kunrath and Spitzer, 1997; Asa and Valdespino, 1998). Several combinations of social pairings and groupings exist and may be based on the individual animals, the situation and on the approval of the MGWRT (Mexican Gray Wolf Recovery Team). Although very few pairs have not been successfully introduced there have been few reports of a resident wolf reacting to a newly introduced wolf as though it were intruding on an established territory. In addition, there are reports of females in this scenario dominating an introduced male, which may have resulted in minimizing the chance for successful breeding (Servín and Lyndaker, 1998).

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Endangered species are by definition rare usually few study subjects are available. Behavioral studies can be an important element to successful captive management of a species. Currently, little information is available on the behavior of captive Mexican gray wolf pairs (Alonso-Spilsbury *et al*., 2000a, b; Escobar-Ibarra *et al*., 2006), which is a social structure successful for many canid species (Moehlman, 1989). According to Mech (1970) in wolf packs only one pair will form a monogamous unit and reproduce.

The present study was aimed to know:

- The social interactions dynamics during the pair bonding establishment of two pairs of wolves in confinement.
- The mating behavior of two captive pairs of wolves.
- The use of space by the wolves in two enclosures.

The observed wolves kept at the Zacango Zoo (ZZ) in the State of Mexico and León Zoo (LZ) in the State of Guanajuato are part of the Mexican Wolf Recovery Plan.

Study Area

The present study took place at two Zoos, the Zacango Zoo located on km 7 of the Metepec-Zoo highway, Calimaya Municipality, State of Mexico and the León Zoo, located in Ibarrilla highway, km 6 in the state of Guanajuato.

Two Mexican gray wolves were observed in each zoo; animals were identified with a microchip. The male called Don Pablo, (McBride lineage; studbook number 429), was eight years old and lived at the Zacango Zoo from 1997 till 2000. The female, called La Güera (San Juan de Aragón lineage; studbook number 88) (Siminski 1998), was transferred from the Ecological Center of Sonora and was relocated to the ZZ on November 1998; she was 9 years old. The Leon pair included El Sapo, from the McBride lineage, a six-year old male coming from Guadalajara and moved to León Zoo in mid Dec. 2001. The female named Gila (San Juan de Aragón lineage; was aprox. 5.5 years old) and has been the resident at León for the last 4 years. As usually in this species, the female has a smaller in size than the male, which made the identification easier, without having to mark the animals.

On their arrival both foreign wolves were housed in an adjacent pen at the back of the residents´ enclosure, separated by a mesh that allowed visual and smelling contact. The ZZ pair had not displayed reproductive behavior before; *La Güera* spent three years with a male at the Ecological Center of Sonora, without showing sexual behavior at all (Cinco, 1998, unpublished). In the case of the LZ pair, the female displayed reproductive behavior several times, though no reproductive success was monitored.

Both exhibits were constructed based on the recommendations of the Mexican Gray Wolf Recovery Team (USFWS, 1982).

Materials and Methods

Behavior Observations

Animals were observed daily from February to April (1999 for ZZ and 2001 for LZ) during the breeding season. In order to obtain information on the wolves' social behavior, a combination of the focal and continuous, and time sampling registration methods was used (Altmann, 1974; Martin and Bateson, 1986; Lehner, 1996). Focal sampling meant observing one individual or dyad for a specified amount of time and recording all instances of some selected behavioral patterns (Martin and Bateson, 1986). Continuous recording, known as all-occurrences recording method provided an exact and faithful record of the behavior, measuring true frequencies (Martin and Bateson, 1986). As we were

Table 1: Sexual behavior of two pairs of Mexican gray wolf in captivity

Behavior	Description
Anus-genital region exposure	The female exposes the anus-genital region standing in front of the male; she then lifts or twists her tail a bit
Smelling and licking the anus-genital region	The male smells or licks the females anus-genital region
Mating	The male lifts one or both front legs on the female's back and hugs her strongly
Hip's movements	After holding the female for a while, the male starts a series of hip movements pushing her
Copulation	Complete penetration; the male pushes with its front members to the back, the tail drops and intense hips and rear member moves occur. The genital lock is then made and penetration occurs

From Servín (1991)

particularly interested in social interactions involving sexual behavior, when the first mating signs were detected continuously recording of all occurrences of selected behaviors and events were monitored from 8:00 to 21:00 h. Observations were interrupted between 9:00 and 10:00 h, meantime the wolves facilities were cleaned and observers took a break. Time sampling method was used from 24:00 to 1:00 h and from 4:00 to 5:00 h, recording observations every 30 min. Data were obtained on both wolves using both direct and videotaped observations. To avoid conflicts with the observations, an order of acts observed was established having a preference for the social over the maintenance and the active over the passive ones.

The wolves were videotaped in their exhibit using a still, infrared, remote control, 360° and zoom video camera (set in the middle of the exhibit), binoculars and a portable camera. Observations were done from one of the animal's empty dorms, or from the dorms' flat roof. All the observations were registered pointing out: the observer, starting time, the giver and the receptor, codified social interactions and maintenance activities and the quadrant where the conduct was displayed.

Behaviors studied were derived from Servín (1991) ethograms. The pre-codified social behaviors used in this study were: agonistic (aggression-defense), neutral (affiliative) play and sexual (Table 1).

Reproductive assistance was provided for the ZZ pair. The breeding status of female wolves is generally not as easily detected, therefore vaginal cytology smears were taken every four days utilizing Olson *et al*. (1984) technique. Electro-ejaculation was assessed using Howard *et al*. (1986) criteria, assisted by Rivera and Jiménez, from the breeding program.

Statistical Analysis

Comparisons before and after AI for each individual and between individuals were performed in order to find changes within periods and animals. Event frequencies for social interactions were analyzed using the Chi square test; in those cases where n were small, probabilities associated with these values were obtained directly from a binomial test table (Siegel and Castellan, 1988).

Agonist behavior was split in 6-15 days periods for the ZZ pair and 3-15 days for the LZ pair. To compare differences between periods the binomial test (Siegel and Castellan, 1988) was performed. The α was set at 0.05 and all tests were two-tailed.

Results

Social Behavior

It is known that dominant wolves eat first and consume a greater quantity of food than their subordinates (Servín, 1984). Our data suggest that the ZZ female was dominant over the male since she fed herself before the male did (93.2 and 85.5%).

Before the AI the ZZ female displayed aggressive behavior in a higher frequency than the male (34 vs 4; $\chi^2 = 11.8421$; df = 1; $p<0.001$); this behavior pattern was not observed after AI took place

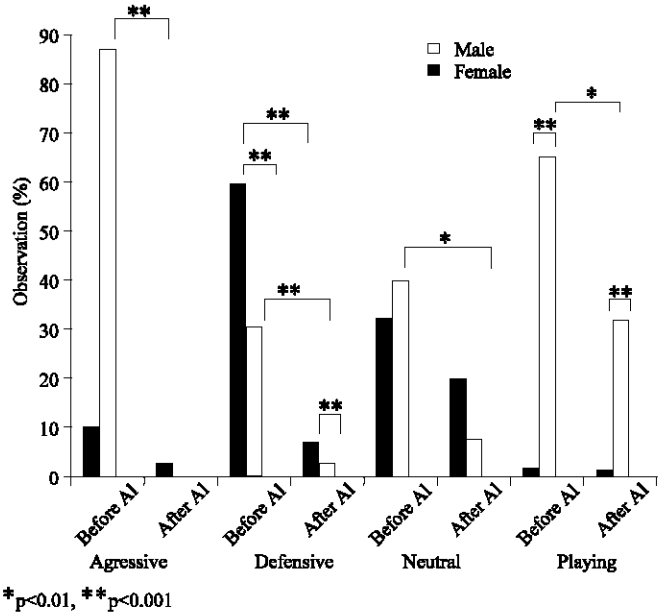

Fig. 1: Frequencies of observed social behavior for the ZZ pair of wolves, before and after Artificial Insemination (AI)

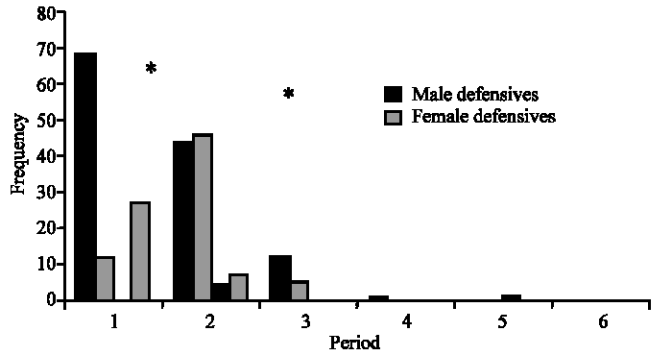

Fig. 2: Frequencies of aggressive behavior in the ZZ pair

(Fig. 1). The male was more defensive than the female before AI (112 vs 58; $\chi^2 = 8.5765$; df = 1; $p<0.01$); however, after the artificial insemination this behavior decreased in both wolves (13 and 5; χ^2 binomial test, $p<0.05$; male and female, respectively). On the other hand, the affiliative (neutral) behaviors were similar before AI, but the female showed a more notorious diminishing after the artificial insemination (5 vs 26; $\chi^2 = 7.1129$; fd = 1; $p<0.01$).

When agonistic behavior was analyzed in 15 days intervals (6 periods), the social integration process became clearer (Fig. 2) for the ZZ pair. Significant differences ($p<0.0001$) were found for defensive and aggression frequencies for the first period. Afterwards, the defensive frequency decreased and remained constant for both animals. Aggressiveness was abolished until the 4th period.

So far, the LZ pair showed no significant differences for the social behavior (Fig. 3). Nevertheless, during the breeding season, differences were observed for playing, the female played more than the male (56 vs 7; $p<0.001$) and sexual activity was more displayed by the male (514 vs. 238, $p<0.001$).

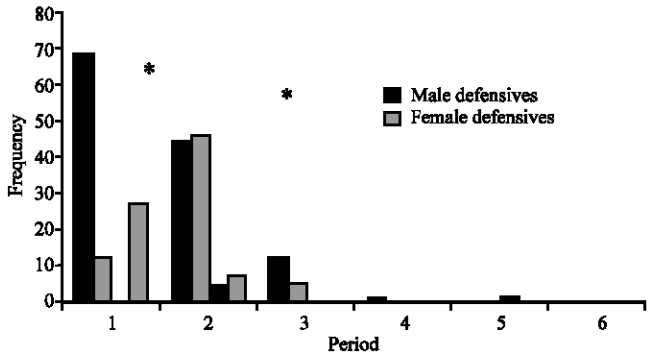

Fig. 3: Frequencies of aggressive behavior in the LZ pair

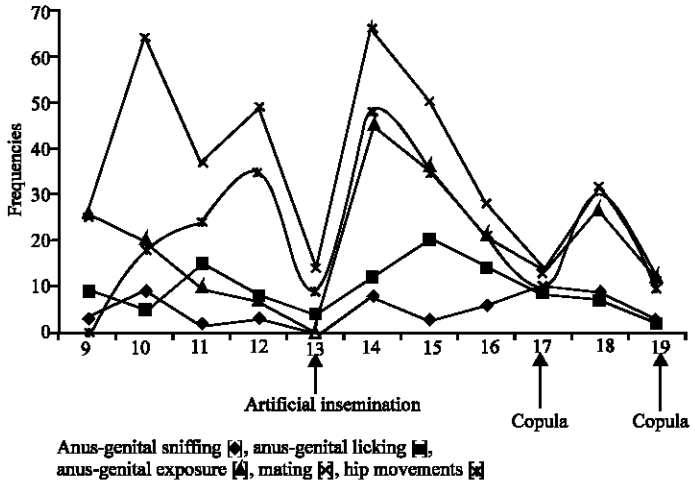

Fig. 4: Frequency of courtship behavior observed in the ZZ pair of wolves during the breeding season

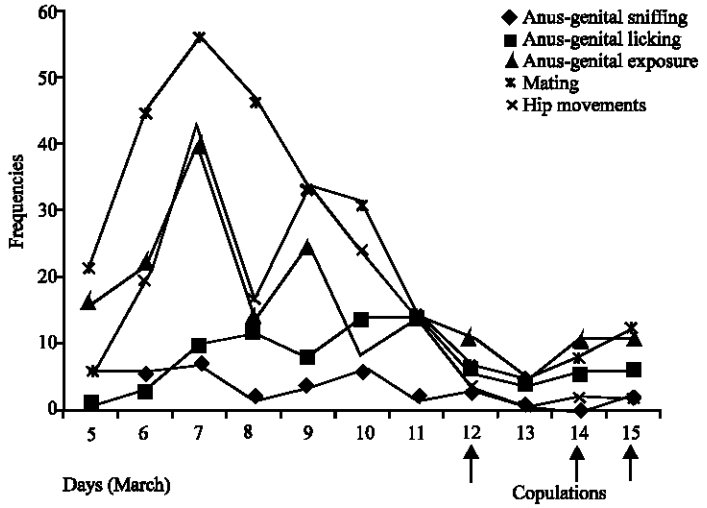

Fig. 5: Total number of courtship behavior observed in the LZ pair of wolves during the breeding season

Table 2. Breeding behavior of two Mexican gray wolf pairs in captivity

Variable	Zacango Zoo (1999)	León Zoo (2001)
Breeding season (days)	11	11
Courtship events (total)	1006	752
Mountings (number)	389	177
Mountings/day (average)	35	26
Total number of copulas	2	3
Average copula duration (min)	20	13

Sexual Behavior

The breeding season of the Mexican wolf captive populations occur from the first week of February through the first week of March (Servín, 1997).

Cornified cell types predominated in the vaginal smears on days 6, 8 and 13 of March, confirming the oestrus stage observed on the female ZZ behavior. No cytology studies were carried on the LZ bitch.

The ZZ female showed her reproductive cycle since mid February; she continue showing proestrus on March 6 and then became sexually receptive, making the male aware of this by presenting her anus-genital region from March 8 through the 24. The male responded to the solicitude by sniffing and licking the female genitals, showing a small rise on this activity by March 15 and 16 (Fig. 4).

A double electro-ejaculation and AI were carried out on March 13, in collaboration with the Binational Committee Reproduction Team for the Recovery of the Mexican Gray Wolf after assessing the vaginal cytology data and the male's behavior showing a lot of mating without copulation. The reason behind was not letting the fertilization opportunity pass by, because this specie is mono-estrus stationary (Mech, 1970; Seal *et al*., 1979; Bernal, 1990a, b).

In the ZZ pair, mating behavior was recorded 389 times during 11 consecutive days, with an average of 35 mounts per day. Mounting pattern was quite variable throughout the cycle. It is important to point out that during the mating season, the day that recorded higher sexual activity was on March 14, with 23 mountings registered in one hour (66 in a day), precisely a day after AI was carried out. Since this time, the sexual activity declined, although a small rise in pelvic movements and mountings was appreciated on day 18 (inter-copulation period).

On March 17 and 19 a natural copulation in the ZZ pair was observed at 17:48 and 17:18 h; the durations were 20 and 17 min, respectively, the locking posture mean duration was 19 min for both.

The LZ pair showed a breeding season of 11 days too, from the 5th to the 15th of March they displayed a total of 752 sexual events (Fig. 5), 177 of them were mountings, with an average of 26 mounts per day. Three copulas were videotaped, two of them lasted 10 min and the other 18 min.

In both pairs, copulation days were characterized by low sexual activity: less solicitude from the female and less pelvic thrusts and mounting from the male. A summary of the mating behavior from the two observed pairs is shown in Table 2.

The Use of Space

For the ZZ pair statistical differences were observed in the use of space the wolves showed a preference ($p<0.0001$) to perform their activities on a reduced place far away from the human transit and especially those related with the courtship behavior.

Although the LZ pair results were not analyzed, a trend was found to use the space far away from human contact too to perform most of their social interactions including the sexual ones.

Discussion

For the ZZ pair the male was more active than the female, he displayed more stereotypic pacing compared with her; he also showed a smaller proportion of resting. Although the enclosure is provided

with the minimal requirements established by the USFWS (1982), this behavior suggests reduced welfare (Monaghan and Wood-Gush, 1990), so improvements through an environmental enrichment program were recommended.

In the Mexican wolf pack the hierarchy is based on social interactions like affiliative, aggressive and submission behaviors that occur in a dynamic system, that is to say, that it suffers changes related to the individnal's development (Servín, 1991). The fact that the ZZ wolves were adult animals, in addition to their pairing housing with no conspecific contact make us believe that the absence of aggressive behaviors, as well as the global decrease of the defensive behavior were the result of a social integration process, which took place between days 45 and 60 after commingling together in the enclosure.

Wolves are seasonal breeders experiencing a single estrus between late January and early April (Seal *et al*., 1979), according to Mech (1970) individnals in lower latitudes generally breed earlier. Our results indicate that the breeding season in the Zacango and Leon pairs was similar to that reported for Mexican gray wolves in captivity (Servín, 1997).

Esquivel *et al*. (1993) showed that proestrus lasts in average 27±6.48 days. Indeed, it is known that wolves may copulate daily for periods of 3 to 15 days (Young, 1944; Murie, 1944; Lentfer and Sanders, 1973; Zimen, 1976). Young (1944) stated that estrus continues for 3 to 5 days in wolves; Seal *et al*. (1979) found that the duration is 9.0±1.2 days, whereas Rivera *et al*. (1993) found 26 days. Our results indicate a breeding period of 11 days for both pairs. According to Asa and Valdespino (1998) the relatively long period of estrus provides time for more copulations.

Our data are similar with Mech (1970) and Servín (1991) who indicate that although much courting takes place throughout the breeding season, only a small percentage of the courtship attempts end in copulation (2.4%). Even though there was a high quantity of courtship events during the breeding season in the Zacango pair, only a minimum percentage of the mating ended with copulation (0.6%). A little increase in copula events was observed for the LZ pair (1.6%) compared with the ZZ pair.

According to Rabb (1968; cited by Mech, 1970) the tie caused by the swelling of the base of the males´ penis, may continue for as long as 36 min, Mech (1970) witnessed it lasted 15 min in the Isle Royale wolves. The Zacango pair data coincides with Bernal (1990b) and Servín (1991) findings in the mean duration of the locking posture, which was around 20 min. For the LZ pair the locking posture lasted 13 min in average.

The wolves´ reproductive performance could be affected by several factors, among them: high rates of noises caused by the music played at the ZZ and vendors and stray dogs closer to the LZ enclosure. Also, vocalizations of their closer neighbors (e.g., lions); management of equipment to condition the exhibit during the reproductive cycle, when a cactus barrier and a tubular structure were located outside the ZZ enclosure, so people did not get too close to the window to watch the animals and probably, the presence of humans somewhat near their enclosure. For small to medinm-sized predators, such as cheetahs and a nnmber of small felid species, it has been reported that housing next to large-sized predators and natural competitors, such as lions and leopards, may decrease reproductive success (Rawlins, 1972; Mellen, 1991).

Unfortunately, neither AI nor copulation resulted in a successful fertilization, we do not know the causes; nevertheless, this could be due to the health problems of the ZZ female when she arrived, or the stress produced by the chemical-surgical handling (entropion correction performed in early February). Another cause could be the estral cycle pursuit, which required restraining her for vaginal cytology every 4 days. On the other hand, manipulations made for two electro-ejaculations and the AI procedures could cause diminishing of the couple's sexual behavior dynamics. There is evidence that restraint procedures constitute one of the most stressful incidents in the life of an animal and intense or prolonged stimulation can induce detrimental responses (Fowler, 1986). Therefore, stress may have

played an important role in the reproductive failure of the confined wolves, as has been reported for captive cheetahs (Mellen, 1991). It is well known for instance, that in wildlife conditions, when a male takes over a lion pride, he kills the young cubs fathered by the previous males (Packer and Pusey, 1983). We do not know if the odors from this carnivore rival at the zoos may induce the wolves not to reproduce preventing implantation to occur.

Conclusions

- Pair bonding establishment took 45 days in the ZZ pair, whereas for the LZ pair, it only took 15 days.
- Both pairs showed a preference to perform their social activities in a quadrant far away from human transit, especially those related to courtship behavior.
- Both pairs showed an intensive mating behavior without reproductive success.
- Due to possible stress related to restraint procedures, concurrent non-invasive physiology studies are urgently recommended to determine the reproductive status of the animals during the breeding season.

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