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Integration of Two Newly Formed Couples of *Canis lupus baileyi* at Two Zoos in Mexico*

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Abstract: The study was done to determine when social integration occurs in newly formed Mexican gray wolf couples. Two wolf pairs, at Zacango Zoo (ZZ) and Leon Zoo (LZ) respectively, were observed daily from the time they were put together until 15 days after the breeding season was over. Social behavior frequencies were split out in five periods: Anterior, Previous, During, After and Posterior to the breeding season. The Binomial test was used to analyze the social interactions between the pairs and the differences between genders. During the reproductive season both couples showed a significative increase in neutral behaviors-mainly by the females, all disappearing in the After period. In both groups, males were on the defensive and playful behavior, aggressive behavior was displayed almost exclusively by the bitches. Again, this last behaviour decreased in the After-breeding period. In conclusion, neither playful nor aggressive behavior observed indicate that wolves had socially integrated, since as in neutral interactions, these behaviors were only shown During the reproductive season and then disappeared afterwards.

Key words: Social integration, *Canis lupus baileyi*, Mexican gray wolf, reproductive season

INTRODUCTION

The Mexican gray wolf (*Canis lupus baileyi*) is listed as an endanger specie under the US Endangered Species Act of 1976 (Hedrick *et al.*, 1997). Currently there are only 89 Mexican wolves in Mexico, all of them born in zoos and wildlife parks in the US and Mexico (Siminski, 2002). According to the Mexican Wolf Recovery Team, wolves are paired a month before the breeding cycle starts; nevertheless, this planning is based on their inbreeding coefficient, without knowledge if the pair bonding and social integration may occur.

The social integration process of various domestic animal species occur at a different time frames. Petersen *et al.* (1989) indicated that the social integration of pigs occurs between the second and seventh week after grouping; Moore *et al.* (1993) pointed out that social integration in pigs is evident 21 days after they mix with other animals. Arnold and Pahl (1974) reported that this process in unknown lambs does not occur until three weeks after they are placed together for grazing. Connell *et al.* (2004) concluded that when increasing rest periods for resident pigs during the first

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weeks after being regrouped, new pigs integrated into the resident group within this time. Regarding wolves, Sthaler *et al.* (2002) observed that a pack accepted a new stud in a lapse of 6 h. At this moment studies on social integration reported in this specie are scarce.

According to Asa (1997, 1999) prolonged pro-estrus in wolf pairs allows for stabilization or reaffirmation of the bond between the couple, it also increases the attractiveness of the female so that the male remains close to her; urine-marking is also increased during this time period mainly by the female (Asa *et al.*, 1990). During the reproductive season the alpha wolf couples set themselves apart from other members of the pack when resting or sleeping (Mech and Knick, 1978; Knick and Mech, 1980). Various studies indicate that the wolf pair bonding is established during breeding (Fox, 1971; Derix and van Hooff, 1995; Rodden *et al.*, 1996). Moreover, Fox (1971) reported that the bond between wolf pairs is hard to see apart from the reproductive period and outside of the reproductive season the social bond of the pack is oriented towards the defense of territory, exclusion of strangers, maintaining the size of the pack and repelling outsiders.

Canine social and reproductive behavior has been documented over the years (Mech, 1970; Packard *et al.*, 1983; Asa *et al.*, 1986; 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 Mexican Gray Wolf Recovery Team (MGWRT). Although very few pairs have not been successfully introduced, there have been a few reports of a resident wolf reacting to a newly introduced wolf as though it were intruding on an established territory. In addition, there have been reports of females in this scenario dominating an introduced male, which may have resulted in minimizing the chance for successful breeding.

The objective of the present study was to determine the time in which social integration occur in terms of the presence of affiliative behavior or the absence of agonistic behavior in newly and already formed Mexican gray wolf pairs.

MATERIALS AND METHODS

Animals and Husbandry

Two Mexican gray wolf pairs were observed from the time they were put together for the first time until 15 days after the breeding season was over.

Pair 1 was integrated by the male called Don Pablo (McBride lineage; studbook number 429); he was eight years old and lived at the Zacango Zoo (ZZ). The female called La Güera (San Juan de Aragon lineage; studbook number 88) (Siminski, 1998) was transferred from the Ecological Center of Sonora and was relocated to the ZZ, she was 9 years old. This pair was housed at Zacango Zoo, located on km 7 of the Metepec-Zoo highway, Calimaya Municipality in the State of Mexico.

Pair 2 was integrated by the female named Gila (San Juan de Aragón lineage; she was about 6 years old) and has been the resident at Leon Zoo for the last 4 years and El Sapo from the McBride lineage, a six-year old male coming from Guadalajara and moved to Leon Zoo (LZ). This pair was housed at the LZ, located in Ibarrilla highway, km 6 in the State of Guanajuato. Wolves were maintained together for two consecutive breeding cycles.

As usually in this species, the female was 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. Both exhibits were constructed based on the recommendations of the MGWRT; a minimum size of 900 m² has been suggested for Mexican wolf breeding facilities (USFWS, 1982).

Behavior Observations

Animals were observed daily from February to April (1999 for ZZ and 2002-2003 for LZ), during the breeding season.

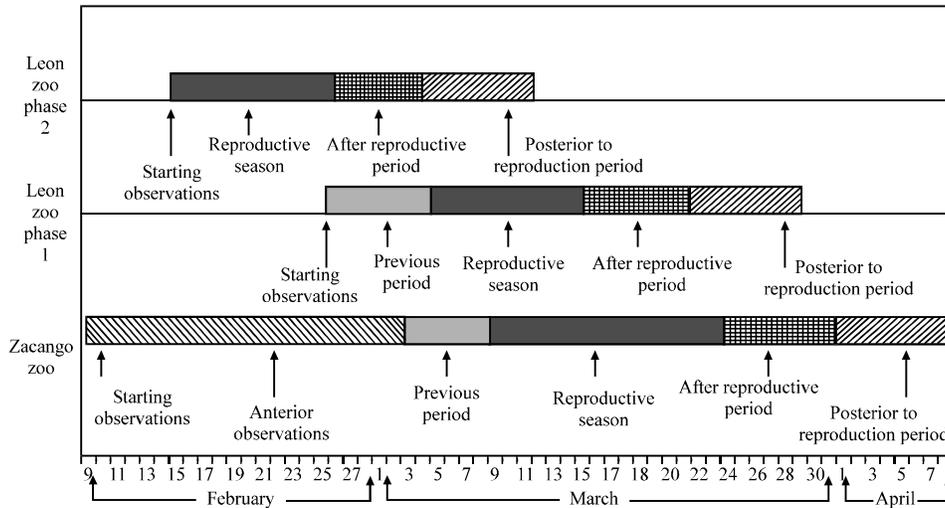


Fig. 1: Observation periods of Mexican gray wolves pairs

In order to gather information on the wolves' social behavior a combination of focal and continuous and time sampling registration methods was used (Altmann, 1974; Martin and Bateson, 1993; 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, 1993). The particular interest of this study was the social interactions involving sexual behavior, therefore when the first mating signs were detected, continuously recording of all occurrences of selected behaviors and events were monitored from 07:00 to 22:00 h, with a resting interval from 15:00 to 16:00 h. Data was obtained on both wolf pairs using both direct and videotaped observations.

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.

Behaviors studied were derived from Servin (1991) ethogram. The pre-codified social behaviors used in this study were: agonistic (aggression-defense), neutral (affiliative) and playing. Social behavior frequencies were split out in five periods, according to the time period observed in Fig. 1: (1) Anterior, (2) Previous, (3) During, (4) After and (5) Posterior to the breeding season. The lasting of the During period was determined through the sexual behavior of the wolves; the other periods were adjusted to 6 days each in order to standardize the time sample. The difference between the After and Posterior periods was that Posterior was farther from the estrus cycle than the After-period.

Statistical Analysis

The Binomial test was used in order to analyze the social interactions between the couples and the differences between genders (Siegel and Castellan, 1988).

RESULTS

Newly-Formed Couples

Neutral Behavior

Six neutral or recognition behaviors were recorded in this study: snout-snout, snout to skin, biting of skin, licking of face, hitting of face and biting of paws.

Regarding the ZZ couple the analysis of the observations using the Binomial test revealed significant differences in the Previous period, the female emitted with more frequency (0 vs. 8; $p < 0.004$) neutral behavior. In contrast, no significant differences were found between neither individual, nor Previous (10 vs. 4; $p > 0.09$; male and female, respectively), nor During (10 vs. 14; $p > 0.419$), nor After (4 vs. 2; $p > 0.344$) the reproductive season; however, it is worth mentioning that in the Posterior period neutral behavior was scant.

The frequency of neutral behavior before the reproductive season for the wolves at LZ was lower in the male compared to the female (1 vs. 6; $p < 0.0625$). During the reproductive season the frequency of the behavior increased significantly more in the female (17 vs. 27; $p < 0.0362$). After the reproductive season all neutral behaviors ceased.

Offensive Behavior

Nine offensive conducts were registered throughout this study: biting of snout, attack and bite, attack, offensive threatening, imposition, persecution, severe bite, escort and threaten.

Statistical differences were found before the reproductive season; at the ZZ the male displayed more offensive behavior than the female (11 vs. 2; $p < 0.011$). However, During reproduction the bitch showed a significant increase in this behavior compared to the male (47 vs. 15; $p < 0.00001$). On the other hand aggressive behavior was abolished after the reproductive season (Table 1). In contrast, the frequency of offensive behavior in the ZL couple was significantly higher in the female than in the male (0 vs. 11; $p < 0.003$) before the reproductive season. In the same way during estrus, emission of offensive behavior was significantly higher in the female compared to the male (14 vs. 39; $p < 0.022$). An interesting finding was that aggressive behavior decreased at the end of the reproductive season (Table 1).

Defensive Behavior

Four defensive behaviors were observed: defensive bite, retreat, defensive threat and maintaining distance. The males in both groups were on the defensive; the male of pair 1 showed more submissive encounters in the period Anterior (12 vs. 0, $p < 0.001$) the reproductive season. Previous to the reproductive season, the male showed more defensive behavior compared to the female (9 vs. 0; $p < 0.141$). Submissive behavior was not observed in the periods During- and Posterior-the reproductive season by either of the wolves at Zacango (Table 1). Regarding the ZL pair almost no submissive behavior was shown ($n = 6$), however the male had a relatively higher number of this type of behavior

Table 1: Frequency of aggressive behaviour encounters in two couples of Mexican gray wolf at Zacango and Leon Zoos

	Observation period				
	Anterior	Previous	During	After	Posterior
Zacango pair					
Submissive male	12**	9	1	0	0
Submissive female	0	1	0	0	0
Aggressive male	19	11**	15*	3	0
Aggressive female	12	2	48	0	0
Leon pair 1st cycle					
Submissive male	-	4	1	0	0
Submissive female	-	0	1	0	0
Aggressive male	-	1	13	0	1
Aggressive female	-	11**	33	3*	2
Leon pair 2nd cycle					
Submissive male	-	0	0	0	0
Submissive female	-	0	0	0	0
Aggressive male	-	0	19	0	0
Aggressive female	-	0	37*	0	2

Binomial test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

Table 2: Frequency of playing and neutral behaviours in two couples of Mexican gray wolf at Zacango and León Zoos

Zacango pair	Anterior	Previous	During	After	Posterior
Playing behaviour					
Male	1	0	3	0	0
Female	33**	41***	46***	16**	5
Leon pair 1st cycle					
Male	-	0	7	0	0
Female	-	3	56***	0	0
Leon pair 2nd cycle					
Male	-	0	1	0	2
Female	-	0	91***	0	17*
Neutral behavior in Leon couple (2nd cycle)					
Male	-	0	66***	3	41**
Female	-	0	19	5	27

Binomial test *p<0.05, **p<0.01, ***p<0.0001

compared to the female Before the reproductive season, although this was not statistically significant (4 vs. 0; p<0.62; respectively). Whereas During the reproductive season both animals had the same number of defensive behavior encounters (1 vs. 1; p>1.00) (Table 1).

Playful Behavior

Three forms of play were observed and quantified: the request for play, play with body contact and persecutory play. The request for play was exhibited more often in both wolf pairs.

Playful behavior was shown almost exclusively by the female wolves; in the case of ZZ pair significant differences were found in the Anterior period, only the female (33 vs. 1; p<0.0001) showed playful behavior. In the period Previous the reproductive season, the female showed more playful behavior than the male (41 vs. 0; p<0.00001). This pattern was similar During the reproductive season (46 vs. 3; p<0.00001). However, the frequency of playful events diminished notably after the reproductive season (16 vs. 0; p<0.0010) (Table 2). In the period After, playful behavior diminished dramatically in the female (5 vs. 0; p<0.031) compared to the male. With regard to the LZ couple, the quantity of playful encounters were less (n = 64) than the ZZ couple; before the reproductive season very little playful behavior was observed and there was no difference between the male and female (3 vs. 0; p>0.125). During the reproductive season there were significant differences and the female showed a larger number of playful events compared to the male (56 vs. 7; p<0.0001). On the other hand, the playful behavior vanished After the reproductive season (Table 2).

Playful body contact and persecutory play were less frequently observed in both couples. However, the wolves at the ZZ showed greater number of events of this type of behavior compared to the LZ wolves.

Established Couple

Neutral Behavior

The Binomial test used in the second phase of observation for the ZL couple, showed that the neutral behavior increased significantly During the reproductive season, the male (66 vs. 19; p<0.00001) showed more neutral behavior than the female. After the mounting period neutral behavior in both animals almost disappeared (3 vs. 5; p>0.363). However in the Posterior period, the neutral behavior increased significantly more in the male (41 vs. 27; p<0.05) compared to the female (Table 2).

Offensive Behavior

No statistical differences were observed in the second cycle of observation in the ZL wolf couple, however the female tended to be more aggressive (37 vs. 19, p<0.0111) compared to the male During the reproductive season (Table 1). After the breeding season, this behavior almost vanished.

Defensive Behavior

Defensive behavior in the LZ established pair did not ever repeat itself in either of the two wolves at any time (Table 1).

Playful Behavior

During the second observation phase the same tendencies were maintained in the LZ couple, the female showed more playful behavior compared to the male. There were significant differences between the male and the female (0 vs. 31; $p < 0.0001$) at the moment of estrus (Table 2); on the contrary after this, playing between the two wolves disappeared completely. However, in the Posterior period playful behavior significantly increased in the female (2 vs. 17; $p < 0.001$).

DISCUSSION

Traditionally in wolves, bonding behaviors consist of the following: double marking, nuzzling, parallel walking and sniffing of the genitals or urine (Harrington, 1982; Escobar-Ibarra *et al.*, 2006). However, in the present study social behavior measured through playing, affiliative and agonistic conducts showed differences only During the breeding season. These results agree with Carter (1998), who states that social behaviors, including social bonds are active processes that involve more than simply a failure to avoid another individual.

Recently-Formed Couples

Neutral Behavior

In both couples neutral behaviors vanished when the reproductive season was over; in fact they were observed only During the breeding season, so it is possible that exploration was used in the endocrine stage so that the pairs could detect an appropriate time for copulating. Neutral behavior in the ZZ pair was more frequent in the male, with exception of the first days together with the female, in this lapse of time she displayed more this type of behavior. During the breeding period neutral behavior was greater in comparison to other periods. Similarly in the LZ pair this behavior was increased significantly during estrus, although the female had more frequent neutral behavior in all periods. The couple in Leon showed the same type of behavior, which increased During estrus, but the female had more neutral behavior in all periods. Our data is similar to what was reported by Servin (1991) in the Mexican gray wolf, he indicates that friendly behavior is more frequent in the winter months. The data is also similar to Rodden *et al.* (1996) regarding the short tailed maned wolf (*Chrysocyon brachyurus*) where friendly behavior increased during mounting periods. The low frequency of friendly behavior between the wolves is probably because the animals did not have enough time together before the reproductive season began; as Bradshaw and Hall (1999) indicated that friendly behavior between cats is less frequent in non-related couples as compared to animals from within the litter. Derix *et al.* (1993) observed that a high level of genital inspection does not only occur during the reproductive season.

Agonistic Behavior

Aggressive behavior from the male at the ZZ was significantly higher Previous the reproductive season as compared to the female. This is similar to the findings of Medjo and Mech (1976), who observed that the alpha wolf is more aggressive and protective than the female. However, our findings do not agree with Creel *et al.* (1997) whose observations in another carnivore, the wild African dog (*Lycan pictus*) indicate that they are less aggressive during breeding.

The frequency of aggressive behavior in the bitch at ZZ increased significantly, similarly the LZ bitch was more aggressive than the male in the period Previous and During estrus. Our data coincides

with Derix *et al.* (1993), who indicated that the alpha female wolf shows a high frequency of dominance before, during and after the breeding period. Packard *et al.* (1985) reported that the males with low levels of testosterone receive more aggression and show less aggressive, sexual and marking behavior. In our study there was an increase in aggressive behavior during estrus, the data is similar to that of Rabb *et al.* (1967), Fox (1973), Harrington *et al.* (1982) and Sands and Creel (2004) in wolves and that of Rodden *et al.* (1996) in the maned wolf. Derix *et al.* (1993) found that the dominance conflicts are initiated particularly by the alpha female wolf and show an increase during the reproductive season. However, reports on the Mexican gray wolf by Servín (1991) are different, indicating that aggressive behavior is shown throughout the year; this does not coincide with Creel *et al.* (1997) either, who found that aggressive interaction diminished during estrus in the African wild dog.

Creel *et al.* (1997) observed that dominant African wild dogs are more aggressive and this is correlated with a high rate of mounting and therefore better access to the high range females. In the case of the wolves in our study, the relation is not similar since none of the males showed a high frequency of aggression and even though they had a high number of mountings, the ZZ wolf was able to attain a complete copulation only two times and the wolf at the LZ showed three copulating lockings. Therefore, the aggressive behavior decrement is not an indicator that the couple had accepted one another, since aggressive behavior should decrease before copulation indicating acceptance. In contrast, aggressive behavior turns out to be part of the courtship of wolves (Rabb *et al.*, 1967) and therefore it is not a good indicator of social integration in this study.

According to Moran (1982) and McLeod *et al.* (1996) submissive behavior is important in order to determine the dominant relationship between wolves. The males in both couples were on the defensive in comparison to the females in both periods of the study. Schenkel (1967) concluded that submission contributes to low range wolves integrating socially, based on its difference in the social hierarchy of the pack. Although female wolves were not considered dominant over the males-further studies must be performed in order to define better this behavior-the findings of the present study indicate that the bitches had more range behavior compared to the males. Therefore, since the defensive behavior is a response to the aggressive interaction, it is not an indicator that the male had accepted the female as a mate, since the agonistic behavior was only shown During the breeding period.

Playful Behavior

Regarding the ZZ pair, the female showed playful behavior with more frequency than her mate during the five periods of the study, more playing was shown During the breeding period, this behavior was dramatically decreased when estrus was over with a tendency to disappear in the Posterior period. Similarly the LZ bitch showed more of this type of behavior than the male Previous and During the reproductive season, in the period After playfulness increased significantly, whereas at the end of the breeding season playful behavior vanished. Present findings coincide with that of Pedersen *et al.* (1990), who observe that the female hyena plays more than the male. Similarly, Zimen (1972) (quoted by Lund and Vestergaard, 1998) indicates that from the time they are pups, playful behavior in wolves is less frequent in male wolves compared to their female counterparts. Present results do not agree with Servín (1991) reports who mentions that playful behavior in the Mexican gray wolf decreases during the reproductive season.

Bekoff (1995) indicates that social play in coyotes is only observed when dominance in the couple has been established. According to Derix and van Hooff (1995) playfulness also establishes a pair bonding in wolves, not only dominance-subordination. Playfulness works to strengthen and form social bondings as well as allowing the animals to acquire communication abilities (Bekoff and Byers, 1981; Barber, 1991). On the other hand, Drea *et al.* (1996) concluded that playfulness in hyena pups can modulate aggression and therefore establish dominant relationships, it also provides a mechanism that allows formation and maintenance of social ties.

Fox (1973) observed that playfulness could be associated with the reduction of stress provoked by dominant adults in the pack. The decrement at the end of estrus in both couples in the present study is probably the reason why aggressive interaction was abolished. However, Medjo and Mech (1976) reported that estrus cycle bleeding was accompanied by high levels of playing in the members of a pack of wolves.

Playful behavior in the case of the two observed couples does not indicate that they have socially integrated, since as in neutral behavior, this behavior was only shown during the reproductive season and then disappeared afterward, with the exception of the ZZ couple that showed very little of ludic behavior after estrus.

Established Couple

Only a second reproductive cycle of the wolves of the LZ could be monitored, the ZZ pair died little time after concluding the newly formed pair study. It turns out difficult to calculate in a second cycle when will the reproductive period initiate; the prior period (Previous) to the mating season could not be monitored in the LZ pair' second cycle, since the estrus in this cycle started within a month in advance, compared with the prior year of observation.

Neutral behavior increased in the second cycle compared to the year before, the male showed more this behavior in each of the periods recorded in the study. However, at the end of the estrus cycle this behavior vanished completely and in the Posterior period it rose again. Agonistic behavior decreased in the second estrus cycle compared to the previous year, although aggressive behavior increased during and at the end of the reproductive season it dramatically decreased. None of the animals showed submissive behavior.

Playful behavior was shown by the female with more frequency during the estrus cycle compared to the male. At the end of the reproductive season playful behavior disappeared completely, whereas in the period after it increased again, the female showed more playful behavior. Our data does not coincide with previous reports by Bekoff and Diamond (1976), who concluded that the lack of play in a coyote couple could be due to the fact that the animals already knew each other and did not have a reason to reestablish or strengthen the bond between them.

In order to form a bond during the mating season it is necessary to select a mate since the bond with the mate can establish or end the sexual bond (Fox, 1971). Boyd and Jiménez (1994) reported that wolves without a mate or helper have less opportunity to successfully raise their young. Even though Mexican wolves are housed in pairs, this does not guarantee the reproduction will be successful; we consider that these animals need to interact with more members of their species in order to allow the female more opportunity to select an adequate male to mate with. Therefore according to our social integration criteria, the LZ couple could have made a bond and show a lot of playful interaction. Unfortunately, observations were not made outside of these periods that could indicate if these behaviors occur during the non-reproductive period.

CONCLUSIONS

Even though our hypothesis stated that affiliative and playful behavior indicate that a couple may form a bond, our data does not indicate this since these behaviors were not present when the breeding season was over. Although neutral and playful behavior increased in the LZ couple in the second reproductive cycle after the breeding period, this data is not sufficient enough to state that the couple had integrated socially. Both pairs at the Zacango and Leon Zoo's could not form a bond, because the newly formed couples were made only few days before initiating the reproductive season and therefore the females could not display the entire behavioral repertoire necessary to make a social bond with the male. However, the LZ wolves that were together all year long do not indicate that they had formed

a bond through their behavior, since their behavioral pattern was similar to that from the previous year. Although it is worth mentioning that the double-marking in the Posterior period (Escobar-Ibarra *et al.*, 2006) could indicate that this couple had formed a bond. In addition to the behavioral observations it is necessary to carry out a hormonal study in order to determine the affiliative behavior involved in the process of social integration in wolves in captivity; as in the case study of prairie vole (*Microtus ochrogaster*) hormones, where it has been determined that vasopressin and oxytocin increase affiliative behavior, these hormones are critical in the formation of pair bonding (Winslow *et al.*, 1993; Cho *et al.*, 1999; Young *et al.*, 1999).

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