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## Scent Marking Around the Breeding Season in Two Newly Formed Mexican Grey Wolf (*Canis lupus baileyi*) Pairs Kept in Captivity

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**Abstract:** The aim of the study was to analyse the scent marking patterns in frequency, type and position throughout the reproductive period of two pairs of *Canis lupus baileyi* housed in two zoos (LZ = Leon and ZZ = Zacango), in terms of newly and established pairs. Focal behaviour sampling was used to register 1,211 scent markings. Data were grouped in three periods: before, during, after and posterior to the reproductive season. Between newly formed pairs no significant difference was found in the previous period ( $U = 6.50, p > 0.065$ ); also, no significant difference was observed in the double marking ( $U = 54.0, p > 0.083$ ) during the reproductive season, although it was greater in the LZ compared to the ZZ pair. After commingling together for one year the established LZ couple, showed an increase in double marking ( $U = 16.5, p < 0.001$ ) during the mating period in comparison with the prior year. Male wolves marked with greater frequency with the leg raised in both, the double and single marking, whereas females marked more in a squatting position. It is concluded that scent marking is different in recently formed pairs in captivity, which are found in a reduced space and have not free choice to elect its mate.

**Key words:** Behaviour, *Canis lupus baileyi*, Mexican grey wolf, reproductive season, Urine marking

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### Introduction

The Mexican grey wolf (*Canis lupus baileyi*) is listed as an endangered species 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.

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Among canids scent marking serves as a mechanism for territory advertisement and enforcement (Peters and Mech, 1975; Rothman and Mech, 1979), as a signal of acquisition and possession of food (Wells and Bekoff, 1981; Harrington 1981, 1982), as an aid in long-distance sex recognition, to promote reproductive synchrony, as internal information to orient members of the resident pack, to express social status and to disperse animals entering occupied territories (Gese and Ruff, 1997). Moreover, double marking between members of the breeding pair could play a role in mate guarding. According to Rothman and Mech (1979), newly formed pairs mark at their highest rates during their first few weeks together, eventually decreasing their rates to those of established packs. Wolf pairs with strong bonds show high rates of double marking, also referred to as simultaneous or alternate-marking, both before and after oestrus (Peters and Mech, 1975; Rothman and Mech, 1979). Perhaps double marking plays a multifunctional role: reproductive synchrony, pair bond behaviour, territory defence and mate guarding. Interestingly, this type of marking is significantly influenced by the social status of the animal and the time of the year (Gese and Ruff, 1997). Behavioural and physiological synchronization of wolf pairs is accomplished through the visual and olfactory stimulus of the double mark; dominant males tend to scent-mark more frequently (Rothman and Mech, 1979).

Four kinds of scent marking had been studied in wolves (Peters and Mech, 1975; Rothman and Mech, 1979; Asa *et al.*, 1985): raised-leg urination (RLU), standing urination (STU), squat urination (SQU) and flexed leg urination (FLU). The different scent-marking postures are strongly associated with the social status of the pack members. In this sense RLU is the most important form of wolf scent marking (Mech, 2003); for instance RLU or FLU result in a higher frequency of urination by the alpha male and female than by subordinates (Asa *et al.*, 1985).

Wolves are seasonal breeders experiencing a single oestrus between late January and early April (Seal *et al.*, 1979). According to Rothman and Mech (1979) and Mech (2003), newly formed pairs probably stay together because they establish a physical and behavioural synchrony during courtship. This synchrony may be stimulated when they scent mark together (double mark). Established pairs' double mark at their highest rates during the breeding season, in fact double scent marking is usually considered indicative of a strong bond in wolves (Rothman and Mech, 1979).

It is considered that wolf pairs with strong bonds show high rates of double marking. The aim of this study was to analyse the scent marking behaviour in frequency, style and posture, performed by two pairs of Mexican grey wolves in captivity, housed together one month before the onset of the breeding season. In addition, the scent marking behaviour displayed by the two pairs of wolves in captivity was compared with other canids living in large enclosures or in free ranging conditions.

#### *Study Area*

Two Mexican grey wolf (*Canis lupus baileyi*) 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 Aragon 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. This pair was housed at the Leon Zoo (LZ), located in Ibarra highway, km 6 in the State of Guanajuato. They were maintained together for two consecutive breeding cycles therefore we had the opportunity to monitor scent-marking behaviour from an established pair without the novelty factor.

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 Mexican Grey Wolf Recovery Team; a minimum size of 900 m<sup>2</sup> has been suggested for Mexican wolf breeding facilities (USFWS, 1982).

## **Materials and Methods**

### *Behaviour Observations*

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

In order to obtain information on the wolves' marking behaviour, focal sampling was used (Martin and Bateson, 1986; Lehner, 1996) from 07:00 to 22:00 h, with a resting interval from 15:00 to 16:00 h, observing every instance where any of the wolves were scent marking; a difference was noted if urine marking was simultaneous or alternated, considering if wolves raised their hind leg or not (Rothman and Mech, 1979).

Marking types monitored in this study were: single marking, when 20 seconds were timed between urine emissions and double marking when both wolves in a pair urine-marked within an interval of 20 sec. It was observed that this marking was frequently shown in less than 10 sec. Data were obtained using direct and videotaped observations. 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 dorms, at the Zacango Zoo, or from a tower at the Leon Zoo.

Scent marking frequencies were split out in four periods: 1) Previous to the breeding season, 2) During the breeding season, 3) After the breeding season and 4) Posterior to the breeding season. The duration of the During period was determined through the sexual behaviour of the wolves; the other periods were adjusted to six days each in order to standardize the time sample. The difference between the After and Posterior periods is that Posterior is farther from the oestrus cycle than the After period.

Daily frequency of double and single scent marking and the total amount of deposited marks was quantified. Double marking implied at least two marks: one of the male and another of the female. Thus, for analysis purposes double marking is referred to an event conformed by two marks, whereas for the single marking event we included the contribution of only one individual in the marking.

Postures were identified according to the gender in: RLU (urinating with one hind leg raised off the ground) and STU (no leg is raised off the ground) for the males, and FLU (one hind leg flexed and lifted slightly under, not to the side of the body, similar to the SQU) and SQU (the back is straight or slightly concave as the hind legs are spread and flexed to lower the anus genital area toward the ground) for the females.

### *Statistical Analyses*

A Kruskal Wallis one way ANOVA test was carried out to look for differences in the total frequency of daily marking among the different periods of observation, for each one of the pairs. In order to establish differences between pairs, the critical value for multiple comparisons was calculated (Siegel and Castellan, 1995).

To determine if the recently formed pairs had the same pattern of marking, Mann-Whitney U-test was calculated, comparing the frequency of scent marking of the couple of ZZ versus the first cycle of the LZ pair. The same test was run to determine if the resident wolves marked more than the foreign ones.

A Binomial test (Siegel and Castellan, 1995) was carried out to analyse the different posture frequencies within periods and animals.

## **Results**

### *Overall Scent Marking*

A total of 1,211 marking events were observed during the three breeding cycles; 567 accounted for the Zacango Zoo pair and 236 and 308, for the Leon Zoo pair during their first and second reproductive cycles, respectively.

### *Zacango Zoo*

Wolves of the ZZ did not show significant differences neither in the frequency of total marking (Kruskal Wallis:  $X^2_2 = 5.92$ ,  $p > 0.052$ ) nor in the double one (Kruskal Wallis:  $X^2_2 = 5.10$ ,  $p < 0.078$ ) in any of the observed periods; although the total frequency showed a clear tendency to be increased after the reproductive season. The single marking analysis revealed significant differences among the analysed periods (Kruskal Wallis:  $X^2_2 = 11.00$ ,  $p < 0.05$ ).

Comparisons between ZZ individuals revealed that total marking of both members of the pair showed a significant increase after the reproductive season, with regard to the previous period (R2-R3 = -9.3, critical value = 7.7820,  $p < 0.05$ ). Similarly, single marking executed by the male showed a significant increase in the after period, which indicates that the increment in the total marking was due mainly to the greater frequency in the single marking and not in the double marking, without the female contribution (Fig. 1a).

### *Leon Zoo (First Cycle)*

In the first cycle of observation of the LZ pair, total marking showed significant differences in the different periods (Kruskal Wallis:  $X^2_3 = 17.60$ ,  $p < 0.001$ ). The results showed an increment during the oestrus, but later on scent-marking decreased significantly in the periods after and posterior. Significant differences (Kruskal Wallis:  $X^2_3 = 11.71$ ,  $p < 0.008$ ) in the double marking were found among the during, after and posterior periods; the highest frequency was found in the during period, whereas double marking disappeared completely after the reproductive season. Regarding single marking, the analysis revealed a significant increment (Kruskal Wallis:  $X^2_3 = 15.50$ ,  $p < 0.001$ ) during the reproductive season and in the posterior period.

Single marking displayed by the male was significantly greater during the mating period in comparison with the after period (R2-R3 = 14.79, critical value = 9.2015,  $p < 0.05$ ), to be increased again in the posterior period, although the values were not significant. The female presented the single marking with little frequency in the previous and during periods', while in the after and posterior periods' marking was not observed at all (Fig. 1b).

### *Leon Zoo (Second Cycle)*

In the second reproductive cycle the LZ pair did not show significant differences in none of the periods of observation neither in the global marking (Kruskal Wallis:  $X^2_3 = 5.36$ ,  $p < 0.068$ ), nor in the double marking (Kruskal Wallis:  $X^2_3 = 2.12$ ,  $p > 0.345$ ). Nevertheless, there was a tendency to increase the total amount of marking that was favoured by the single marking of the pair, showing significant differences (Kruskal Wallis:  $X^2_3 = 7.40$ ,  $p < 0.025$ ) among the different observed periods.

Comparisons among periods revealed that the male single marking was significantly greater in the during (R2-R3 = 10.5, critical value = 4.7880,  $p < 0.05$ ) and posterior periods (R3-R4 = -7.7, critical value = 4.8079,  $p < 0.05$ ), compared with the after period. Whereas the LZ female presented low, but significantly greater frequency in the single marking during the oestrus (R2-R3 = 9.91, critical value = 4.7880,  $p < 0.05$ ) in contrast to the after period which was totally broken down and with the posterior one in which it appeared again without being barely noted (Fig. 1c).

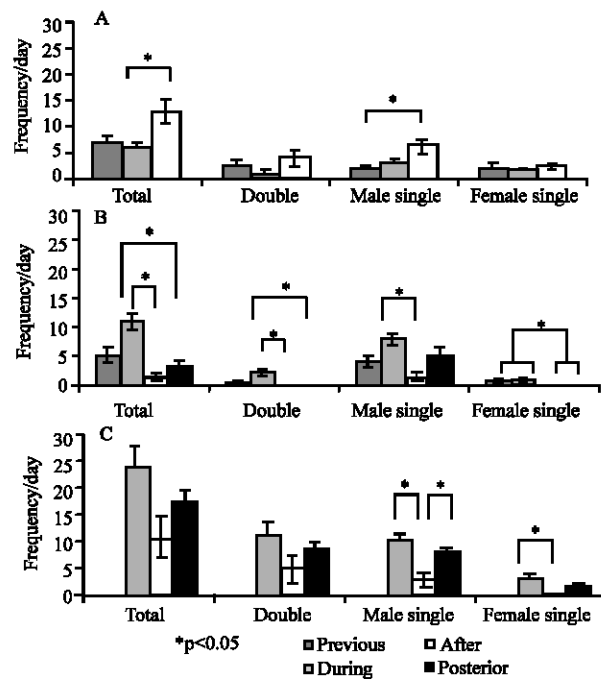


Fig. 1: Daily frequency of double and single marking for every analyzed period. A) Zacango zoo pair, B) Leon zoo pair, C) Leon zoo pair on the second year

*Newly Formed Pairs Scent Marking*

Between newly formed pairs no significant difference was found in the previous period ( $U = 6.50$ ,  $p > 0.065$ ) even though the ZZ couple showed a greater frequency of double marking, whereas the single marking frequency was the same for both pairs ( $U = 14.5$ ,  $p > 0.589$ ) (Fig. 2a). Also, no significant difference was found in the double marking ( $U = 54.0$ ,  $p > 0.083$ ) during the reproductive season, although it was greater in the LZ wolves than in the ZZ pair. Nevertheless, LZ wolves showed a significant increase in the single scent marking ( $U = 30.0$ ,  $p < 0.003$ ).

No differences were observed ( $U = 6.0$ ,  $p > 0.065$ ) in the double marking in the after period, although there was a clear tendency to disappear in the LZ wolves at the end of the reproductive season compared with the ZZ wolves. The analysis of the single marking revealed significant differences ( $U = 0.00$ ,  $p < 0.002$ ) between both pairs, being greater in the Zacango wolves (Fig. 2a).

*Established Pair Scent Marking*

In order to look for differences due to long-term social dynamics, scent marking performed by the same couple (LZ) in two consecutive reproductive cycles, was compared. Unfortunately, the breeding season of the second cycle started before we expected, therefore we could not compare the previous period, but during, after and posterior periods are contrasted in this section.

During the mating period, double marking significantly increased in the second cycle ( $U = 16.5$ ,  $p < 0.001$ ); nevertheless, the frequency of single marking did not show differences ( $U = 37$ ,  $p > 0.079$ ), in spite of being greater in the second year.

The after period did not show significant differences in both double- ( $U = 15$ ,  $p > 0.699$ ) and single marking ( $U = 16.5$ ,  $p > 0.818$ ). Scent marking frequency in this period was clearly smaller than in the previous period in both cycles and for the two types of marking (Fig. 2b).

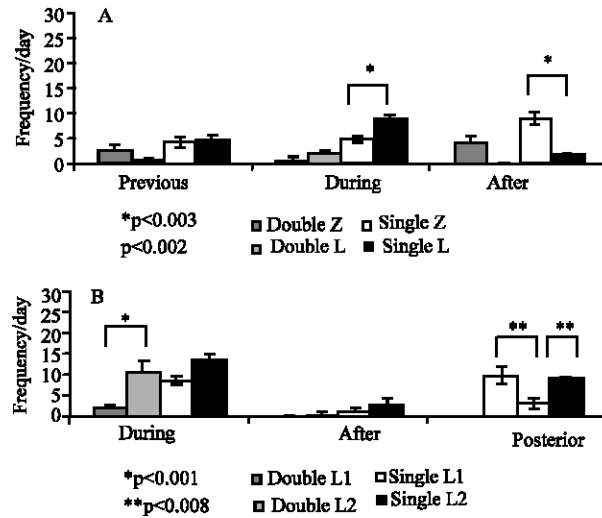


Fig. 2: Daily frequency of double and single marking in newly formed and established pairs. A) Newly formed pairs, B) Established pairs. Double Z: Double-marking in the Zacango zoo pair; Double L: Double marking in the Leon zoo pair; Single Z: Single marking in the Zacango zoo pair; Single L: Single marking in the Leon zoo pair. Double L1: Double marking in the Leon zoo pair in the first reproductive cycle; Double L2: Double marking in the Leon zoo pair in the second reproductive cycle; Single L1: Single marking in the Leon zoo in the first reproductive cycle; Single L2: Single marking in the Leon zoo pair in the second reproductive cycle

Finally, the analysis of the posterior period, revealed that double marking was increased significantly during this period ( $U = 0.000$ ,  $p < 0.008$ ) in the second year, in comparison with the prior year in which the LZ wolves did not show double marking. With regard to the single marking, also a significant frequency increase was observed ( $U = 0.500$ ,  $p < 0.008$ ) in the second cycle (Fig. 2b).

**Marking Postures**

The animals exhibited different types of positions in the double- and single- scent marking for each one of the analysed periods.

In newly formed pairs (Table 1), the Zacango male performed indistinctly both positions (RLU and STU) for the double marking in the different periods. For the single marking there was a difference ( $p < 0.05$ ) showing preference by marking with the leg raised in the after period. Whereas the bitch marked in a squat position with the flexed leg in the double marking; this frequency was significantly greater in the after period ( $p < 0.05$ ). In contrast, for the single marking she combined both positions (FLU and SQU) along the periods.

The LZ male marked significantly more ( $p < 0.001$ ) with the leg raised in both, double- and single-marking in every period of analysis (Table 1); in fact he almost never marked in the other position. In contrast, the female carried out the single- and double marking in both positions before, during and after the reproductive season, the squatting position frequency was significantly greater during the mating period ( $p < 0.05$ ).

After commingling together for a year the wolves of the LZ marked more frequently compared with the first year in both double- and single- marking. In relation to the positions, the male continued with his preference of using RLU ( $p < 0.001$ ), avoiding the STU position. The female combined her positions in the during and posterior periods with a greater significant difference showed in the FLU position in the double marking (Table 2).

Table 1: Different postures of double and single urine-marking frequencies by newly formed wolves

Zacango zoo pair								
Periods								
Wolves	Marking type	Posture	Before	p-values	During	p-values	After	p-values
Male	Double	RLU	3	NS	3	N.S.	8	NS
		STU	5		5		5	
	Single	RLU	7	NS	26	N.S.	26	p<0.05
		STU	5		21		12	
Female	Double	FLU	6	NS	6	N.S.	9	p<0.05
		SQU	2		3		2	
	Single	FLU	8	NS	11	N.S.	10	NS
		SQU	5		15		5	
Leon zoo pair								
Male	Double	RLU	2	NS	15	p<0.001	0	NS
		STU	0		0		0	
	Single	RLU	23	p<0.001	86	p<0.001	9	p<0.001
		STU	1		7		0	
Female	Double	FLU	1	NS	3	p<0.05	0	NS
		SQU	0		9		0	
	Single	FLU	1	NS	1	p<0.05	0	NS
		SQU	3		9		0	

RLU: Raised Leg Urination, STU: Standing Urination, SQU: Squat Urination, FLU: Flexed-leg Urination, NS: Not Significant

Table 2: Different postures on double and single urine-marking frequencies by a the established Leon Zoo wolf pair

Periods								
Wolves	Marking type	Posture	During	p-values	After	p-values	Posterior	p-values
Male	Double	RLU	59	p<0.001	2	NS	28	p<0.001
		STU	0		0		0	
	Single	RLU	109	p<0.001	16	p<0.001	39	p<0.001
		STU	0		0		1	
Female	Double	FLU	48	p<0.001	1	NS	18	p<0.001
		SQU	10		0		3	
	Single	FLU	16	N.S.	1	NS	6	p<0.05
		SQU	17		0		0	

RLU: Raised Leg Urination, STU: Standing Urination, SQU: Squat Urination, FLU: Flexed-leg Urination, NS: Not Significant

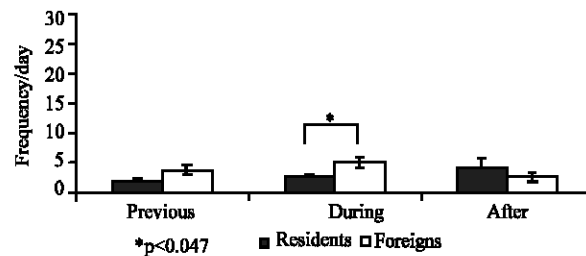


Fig. 3: Daily marking frequency in resident and foreign wolves according to the different observation periods

*Residents Vs. Foreigners Scent Marking*

In order to look for differences between the resident and the foreign wolves, scent marking was compared in every period. It is important to indicate that the foreign wolves were the female for the Zacango Zoo pair and the male for the Leon Zoo pair.



No significant differences were found ( $U = 42.0$ ,  $p > 0.089$ ) in the previous period in relation to the total marking frequency, but the foreign wolves emitted more marks than the residents. The analysis revealed that the foreign wolves marked significantly more than the residents ( $U = 250.5$ ,  $p < 0.047$ ) during the mating period. Curiously, in the period after, the marking frequency showed the same pattern as the previous one without a significant difference ( $U = 64.5$ ,  $p > 0.671$ ) between residents and foreigners, although a tendency to be greater in the posterior period was observed for the resident wolves (Fig. 3).

## **Discussion**

### *Overall Scent Marking*

According to Rothman and Mech (1979), males that obtain a couple show a greater frequency of marking before the onset of the mating season. Present findings do not agree with this study since neither male showed high frequencies of scent marking before the breeding season, in spite that they were housed with their correspondent mate weeks in advance before the oestrus onset. Moreover, marking patterns showed to be different among the observed pairs for every analysed period. The ZZ pair showed a similar scent-marking pattern as reported previously by Rothman and Mech (1979) and Barrette and Messeier (1980) who observed no significant increase in the marking rate during the breeding season in both wolves and coyotes (*Canis latrans*), respectively.

On the contrary, the LZ pair showed a significant increase in scent marking frequency during oestrus. This result coincides with Asa *et al.* (1985) who found that the frequency of urine marking was higher just before and during the breeding season.

Double marking frequency in the ZZ pair was lower during the reproductive season compared with the previous period and it was higher at the end of the oestrus; on the other hand in the LZ pair, double marking during the reproductive season was greater with regard to the prior period and was not observed concluding the oestrus cycle. In contrast, in the second cycle the LZ pair' double marking frequency was higher during the reproductive season and although it diminished in the following periods, the frequency continued to be high in comparison with the first cycle and with the ZZ pair frequencies.

According to Rothman and Mech (1979), the rate of double RLU'ing decreases as duration of the pair bond increases. LZ pair results' indicate that double marking decreased within time for the first analysed cycle, whereas it increased after the breeding season on the second cycle; this could be explained by Rothman and Mech (1979) findings on persistence of marking apparently depending on pair members remaining together and Gese and Ruff (1997) results who state that double marking between members of the breeding pair could also play a role in mate guarding.

Overall, the scent-marking pattern observed in the posterior period could be related to territory defence, as indicated by Roberts and Dunbar (2000) in monogamous antelopes (*Oreotragus oreotragus*). Wirant and McGuire (2004) concluded that urination in female dogs functions in scent marking as well as in elimination, even when females are not in oestrus. Also, major changes in marking rates in coyote occur before and after the breeding period (Wells and Bekoff, 1981).

The two males of this study marked significantly more frequently than the females for every analysed period; results are similar to those reported for domestic dogs and other mammals (Bekoff, 2001; Pal, 2003; Wyatt, 2003). Nevertheless, our data are not coincidental with Asa *et al.* (1990), who found seasonal increases in urine marking in dominant wolf bitches mediated by testosterone. Similarly, Porton (1983) observed that compared to males, female bush dogs (*Speothos venaticus*) made greater use of olfactory signals to advertise both reproductive and social status when breeding pairs are housed.

#### *Newly Pair Formation Scent Marking*

Rothman and Mech (1979) observed that newly formed wolf pairs marked at highest rates during their first weeks together; seemingly, Porton (1983) found high rates of urine marking during initial encounters of unfamiliar male and female bush dogs. Also, in both wild dogs (*Lycan pictus*) and golden jackals (*Canis aureus*), double marking occur almost immediately after members of the pair first met (Golani and Keller, 1975; Frame and Frame, 1976). Our results are contrasting with these findings, scent marking was observed with low frequency in the two observed pairs. This situation could be due to the fact that the LZ pair was formed six days before the onset of oestrus and the ZZ wolves were joined together 30 days before oestrus onset. The mean length of pro-oestrus in wolves is approximately six weeks, with a wide range of 15 to 45 days (Seal *et al.*, 1979; Asa and Valdespino, 1998); interaction time for the wolves in the present study was lower in contrast. During pro-oestrus, as distinguished from oestrus, the female is receptive to mating, hence is attractive to males (Bekoff and Diamond, 1976; Asa, 1997).

Rothman and Mech (1979) found that single marking rates in females in pro-oestrus was greater than the male frequencies. On the contrary, our data do not coincide, the two females showed lower marking rates than the males in the previous period to the mating phase.

It is important to state that 30 days previous to the union of the ZZ pair, the female was isolated in a small pen adjacent to the male one and she could establish visual and olfactory contact only with him, then it could be possible that they have the opportunity to obtain synchronicity, although we could not evaluate this period. According to Bekoff and Diamond (1976) and Porton (1983), urine marking as a major component of bush dog pre-copulatory courtship, probably serves not only to inform pair mates but also to facilitate a sustained state of arousal and inter-activeness culminating in successful breeding. Nevertheless, the two bitches in this study failed to get pregnant although a lot of sexual activity was observed, with only few copulas (ties) monitored (Alonso-Spilsbury *et al.*, 2002).

There was a rise in the marking frequency of both pairs during the mating season, LZ pair showed a higher rate compared with ZZ pair. This result is similar to previous reports by Porton (1983) and Bernal and Packard (1997), who observed high frequencies of scent marking in *Canis lupus baileyi*, related to the sort of substrate and vegetation, but without indicative of a strong pair bond pattern.

If double marking function is information exchange necessary for courtship and mating, such as synchronization, oestrus announcing and pair bonding reinforcement in newly formed canid pairs (Rothman and Mech, 1979; Porton, 1983; Bernal and Packard, 1997; Harrington *et al.*, 2000), then high rates of urine marking should be expected to occur during initial encounters of unfamiliar wolves and marking should decline during courtship or oestrus (Rothman and Mech, 1979; Porton, 1983). The ZZ pair results agree with these findings, but not the LZ pair data, which double marking rates were reduced in the previous period. On the other hand, double marking was significantly high during the oestrus in the LZ pair, in contrast with the ZZ wolves that showed low frequencies of this type of marking, this result coincides with Harrington *et al.* (2000), who mention that double marking is increased during the courtship. After the reproductive season double marking rate diminished in the LZ wolves, which agrees with Porton (1983) observation with bush dogs, who observed that the sequence of marking diminished when the female was fertilized; although the Leon female did not get pregnant, the pattern was similar. Whereas the wolves of ZZ increased their double marking at the end of the oestrus, coinciding with Rothman and Mech (1979).

#### *Established Pair Scent Marking*

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 (Before) to the mating season could not be monitored in the LZ pair's second cycle, since the oestrus in this cycle started within a month in advance, compared with the prior year of observation.

During the second oestrus of the LZ pair it was expected that the single marking remained in basal levels, if it had the function to maintain the territory; nevertheless, this type of marking was increased during the mating period compared with the prior year. Results are similar to Porton (1983) study with high rates of marking during the mating period observed in established couples of bush dogs. For the established pair, single marking rate was increased in the subsequent period to the oestrus in both cycles; although it was greater in the second year, this result agrees with Pal (2003) who observed that marking was increased in those females that have delivered a litter. Nevertheless, as already indicated, in none of the two cycles monitored the female got pregnant.

At this moment we do not know what is the function of the single marking after oestrus; however, we speculate that scent marking after the breeding season has territory defence and maintenance functions, as observed elsewhere (Johnson, 1973; Richardson, 1991; Sillero-Zubri and Macdonald, 1998; Brashares and Arcese, 1999; Roberts and Dunbar, 2000; Bekoff, 2001; Wirant and McGuire, 2004).

According to Rothman and Mech (1979), the rate of double RLU'ing decreases as duration of the pair bond increases. Nevertheless, present results show that after being together for one year, double marking in the LZ pair was significantly increased during oestrus. At the moment, there are no reports indicating the role this kind of scent marking has when the pair is already formed; functions that have been assigned are: defence of territory, synchronization of the oestrus and strengthening of the pair bonds (Rothman and Mech, 1979; Gese and Ruff, 1997; Allen *et al.*, 1999; Harrington *et al.*, 2000). We suggest that one explanation might be that the female is trying to strengthen her relationships with the male threatened by other neighbouring canids, this interpretation is based on the housing conditions of the LZ pair, which consisted of an adjoining pen with another female wolf and an uncultivated area that was visited by stray dogs.

#### *Residents Vs. Foreigns' Scent Marking*

Foreign wolves marked at a higher frequency before and during the mating period, compared with the resident wolves, this result does not agree with previous observations from Gese and Ruff (1997), who found that transient coyotes scent-marked at a lower rate than members of a resident pack did; female transients coyotes performed SQU urinations, but male transients were not observed to show RLU; instead, they displayed either STU or FLU. In contrast with our results, in that study the foreign male marked with flexed leg in a higher frequency and the female used both positions, although she marked more in SQU position with the flexed leg.

According to Sillero-Zubiri and McDonald (1998) resident Ethiopian wolves (*Canis simensis*) are more tolerant of opposite-sex than same sex neighbours. This could explain the low marking rates from the resident wolves of the present study, although the association between the resident and the defended area may depend heavily on territory size, the frequency of territorial encounters and the defensive behaviour of the resident, as explained by Richardson (1991).

Although the sample size in this study was small; we show evidences that scent marking is different in recently formed pairs in captivity that are found in a reduced space and have no freedom to elect their mates, in the periods of oestrus and after this. Moreover, marking was increased when a pair has been already established. In the case of single marking observed before the mating season, our observations coincide with Peterson *et al.* (2002) who report that the female scent marks may act as a mechanism for reproductive synchrony between the female and male, announcing the male they are receptive. In the present study scent-marking was found to be connected with several factors related to breeding, hence it is possible that it is important to the success of courtship in new pairs and

to reproductive synchrony in established pairs. The scent marking previous to the mating is necessary to synchronize the male and to increase the testosterone levels, as suggested by others (Rothman and Mech, 1979; Asa *et al.*, 1990). In other cases it is not confined to the breeding season and therefore some function unrelated to breeding are implied (Johnson, 1973).

According to Rothman and Mech (1979), Porton (1983), Asa *et al.* (1986) and Peterson *et al.* (2002), urine marking appears to have an important function in the formation and maintenance of the pair bond, therefore, marking should decrease when wolves are accepted as a family. We are in disagreement with this function, since the acceptance of the pair cannot be defined only with a decrease of the marking along the time. On the other hand, the bonds are not static and once established they remain the same forever; on the contrary, affiliate bonds and their maintenance may require interactions to strengthen them. Double marking may be a good candidate, since marking in a pair can indicate territory defence, besides guarding the couple.

Overall we consider scent marking has different functions according to the different physiological stages of the animals, for instance, we propose that marking out of the breeding season may be associated with pregnancy signalling in females, pair bonding, litter protection and territory maintenance. Moreover, the couple has to work as a group, in contrast to Wells and Bekoff (1981) suggestion on marking by male coyotes associated with courtship, breeding season, group leadership and aggressive encounters, whereas in the females it is associated with the acquisition and possession of food and with the breeding period. Thus, it is necessary to carry out a more detailed study of the chemical analysis of the urine in the double marking and its association with the different endocrine status of the pair; the results would clarify better the function of the single and double marking. This is the first systematic study on the marking behaviour of Mexican grey wolf in captivity. Further studies involving scent marking throughout a year including non reproductive periods, along with a study of social interactions in confined wolf pairs living in a limited space and forced to accept each other as mates will help to determine if pair bonding has been established.

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