RESEARCH ARTICLE

Cross-Fostering in Gray Wolves (*Canis lupus lupus*)

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Cross-fostering in canids, with captive-bred pups introduced into endangered wild populations, might aid conservation efforts by increasing genetic diversity and lowering the risk of inbreeding depression. The gray wolf (*Canis lupus lupus*) population in Scandinavia suffers from severe inbreeding due to a narrow genetic base and geographical isolation. This study aimed at evaluating the method to cross-foster wolf pups from zoo-born to zoo-born litters. The following was assessed: female initial acceptance of foster pups, growth rate in relation to age difference between foster pups and pups in recipient litters and survival over the first 33 weeks. The study included four litters added by two foster pups in each. The age differences between the foster pups and the recipient litters were 2–8 days. After augmentation, all four females accepted the foster pups, demonstrated by her moving the entire litter to a new den site. Growth rate was dependent on the age difference of the pups in the foster litters, with a considerably slower growth rate in the 8 days younger pups. However, these pups later appeared to be at no disadvantage. Foster pups had a higher survival rate than females' pups, however, the causes of death were probably not kin or non-kin related. The results indicate that cross-fostering works in gray wolves and that this might be a plausible way to increase genetic variation in the wild population. Zoo Biol. 9999:1–6, 2015.

Keywords: *Canis lupus*; wolf conservation; cross-fostering; pup growth rate; pup survival

INTRODUCTION

The gray wolf (*Canis lupus*) was once the world's most widely distributed mammal, but as a result of widespread destruction of habitat and deliberate persecution to mitigate predation on livestock and due to culturally caused fear and hatred, wolves became extinct in much of Western Europe and parts of the contiguous United States. Today, several wolf populations are seriously threatened at a regional level. In Scandinavia, the nominate subspecies European wolf (*Canis lupus lupus*) is considered to be endangered (Large Carnivore Initiative for Europe, 2007). The population is founded by only five individuals, which over 30 years has resulted in a grave loss of genetic diversity and severe inbreeding. Most individuals born after 1997 have an inbreeding coefficient close to a level corresponding to full sibling mating (Liberg et al., 2005). The average inbreeding coefficient in 2010 for breeding pairs' offspring (regardless of number of offspring) was 0.26 (Åkesson, unpublished report, 2011).

Natural immigration from the Finish-Russian populations is hampered because wolves are not allowed to establish territories in the northern half of Scandinavia, because of problems associated with reindeer management. Direct relocation of wild adult specimens, i.e., from the Finnish or Russian populations, is complicated by the requirement of 6 months rabies quarantine, which is not practical to implement with wild wolves. Cross-fostering, i.e., the rearing of non-maternal young by surrogate parents, with zoo-born pups might be an alternative method to introducing new genes into a wild population. This would make it possible to enhance genetic diversity and lower the risk of inbreeding (Macdonald, 2009).

Cross-fostering has been carried out experimentally in Coyotes (*Canis latrans*) (Kitchen and Knowlton, 2005) and as part of Species Survival Plan (SSP) management program

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in Red Wolves (*Canis rufus*) (Waddell et al., 2002), where the efficacy of introducing pups into existing litters (augmentation) as well as completely switching litters (replacement) was assessed. Another attempt to cross-foster two captive-born red wolves into a wild pack was performed in the framework of the Red Wolf SSP in 2002. The pups were augmented into a wild litter at 10 days of age and they survived into adulthood. As yearlings, they helped raise the pack's next litter (US Fish and Wildlife Service, 2004).

The aim of the present study was to examine if cross-fostering captive-to-captive works with European Gray Wolves (*C. lupus lupus*). This is, to our knowledge, the first time the method was tested on European wolves. The following were assessed

a) Female acceptance of foster pups,
b) Growth rates of foster pups and female's own pups, and
c) Survival rates of foster pups and female's own pups.

MATERIALS AND METHODS

Subjects

The study was conducted from 2011-05-23 until 2011-12-31, and included all 43 pups born in seven litters in Scandinavian zoos in 2011. Eight pups were foster pups and 35 pups stayed with their biological mother (throughout this study, pup is defined as being <7 months of age). Three litters were only source litters of foster pups, three litters were only recipient litters of foster pups, and one litter was both source and recipient litter of foster pups. Number of pups, pack sizes, and enclosure sizes are presented in Table 1. Source and recipient litters and age differences are presented in Table 2. The use of control litters had to be dismissed because unusually few litters were born in Scandinavian zoos in 2011, and as many test litters as possible were preferred.

The foster pups and the females' own pups were weighed at the time of augmentation, and on two more occasions in the litter with −8 days age difference and once more in the litter with +3 days age difference. In the litter with −2 days age difference, the pups could not be weighed again because the staff feared that such a disturbance would cause stress and social turbulence in the pack that might risk the survival of the pups. In the litter with −5 days age difference, the pups could not be weighed again since they disappeared (see Section *Survival rates*).

This aspect of the study therefore focused on analyzing how competition between foster pups and the female's own pups may have affected the foster pups' growth rate at two age differences, −8 days and +3 days.

The pups in the litter with −8 days age difference were weighed when the foster pups were 4 days, 16 days, and 26 days of age and their stepsiblings were 12 days, 24 days, and 34 days of age, respectively.

The litter with +3 days age difference was weighted on two occasions—when the foster pups were 5 days of age and 38 days of age and their stepsiblings were 2 days of age and 35 days of age, respectively.

Experimental Set-Up

The foster pups were removed from the source litters at 4–6 days of age. Thus, they had received colostrum before being parted from their biological mother. All pups were taken out from the den and examined clinically by the veterinarian at each zoo. All the foster pups received subcutaneous fluids (9–25 ml RA/NaCl/glucose) to minimize dehydration during the transport. All pups got microchip implants for identification injected subcutaneously between the shoulders, and DNA samples (blood and/or hair) were taken to facilitate future identification. A medium-sized male and a medium-sized female were taken from each source litter. The pups were transported in a dog kennel transport box where they were embedded in blankets with warm water bottles. The foster pups were transported by car in Sweden and by a small airplane in Norway (maximum transport time was 6 hr, minimum transport time was 10 min). The pups were not handled during transport and they were only sparsely monitored to avoid unnecessary disturbance.

Upon arrival at the new zoo, both the foster pups and the receiving female's own pups were examined clinically by the zoo veterinarian (ID-check, weight, and body temperature). All foster pups had more fluid (RA/NaCl/glucose) injected subcutaneously to keep them from getting

TABLE 1. Pack sizes (adults and yearlings) and enclosure sizes in the packs where pups were born in Scandinavian zoos in 2011

	Pack Size				
	Adults		Yearlings		
Zoo	♂	♀	♂	♀	Enclosure Size (ha)
Nordens Ark (NA)	1	1	2	4	1.4
Orsa Björnpark (OB)	1	1	2	2	5
Järvzoo, Pack 1 (JZ1)	1	1	3	2	0.47
Järvzoo, Pack 2 (JZ2)	1	1	3	2	1.2
Kristiansand Dyrepark (KR)	1	1	5	1	2.3
Namsskogans Familiepark (NF)	1	1	7	4	0.8 → 1.2
Polar Zoo (PZ)	1	1	–	–	2.5

TABLE 2. Source and recipient litters of foster pups, total foster litter size and age differences

	Source Litter Size		Number of Foster Pups			Recipient Litter Size			
Source Zoo	♂	♀	♂	♀	Recipient Zoo	♂	♀	Total Foster Litter Size	Age Difference Foster vs. Recipient
JZ1	3	2	1	1	JZ2	3	2	7	−8
NF	4	2	1	1	PZ	4	4	10	−5
KR	3	2	1	1	NF	3[a]	1[a]	6 (4+2)−(1+1)+(1+1)[a]	−2
NA	6	2	1	1	OB	2	4	8	+3

[a]The litter in Namsskogans Familiepark (NF) was both source litter and recipient litter: Two foster pups were added and two of the females own pups were removed from the litter as foster pups to Polar Zoo (PZ).

dehydrated if the receiving female would delay the return to the den. The foster pups were marked with white color (Tippex) on the tail to facilitate later visual identification on game camera images. The females' own pups were stimulated to urinate and all pups in the recipient litter, including the foster pups, were rubbed with urine to give them the same scent. All pups were consistently handled with surgical gloves. The recipient dens were three natural wolf-dug dens and one artificial structure.

Assessments

A Game Camera (BolyGuard SG560B) was mounted outside each of the dens of the recipient litters to detect if the females moved both the foster pups and their own pups after the disturbance, or if they discriminated between the pups. All pups were weighed on irregular occasions until they were too mobile to easily be captured, except the pups in two foster litters, which could not be weighed again due to mortality and management reasons. Survival of the foster pups and the females' own pups in the seven litters were monitored until the pups were 33 weeks of age. Deaths were reported regardless of cause of death.

Statistical Analysis

Statistical analysis were done using SPSS Statistics version 19.0. Results are presented as mean ± SD. The level of significance was set at $P < 0.05$. Survival for foster pups in reference to pups that stayed with their biological mother, and for foster pups in reference to pups born in zoos 2000–2011, were compared with χ^2 Test. Due to the small sample size, no other comparative statistics were computed. The sampling came from all Scandinavian zoos holding wolf litters in 2011.

Ethical Note

The study was performed according to a protocol approved by Linköpings djurförsöksetiska nämnd (regional ethical committee) in Sweden (Dnr 25-11) and Mattilsynet Forsøks-dyrutvalget in Norway (Norwegian Animal Research Authority; Dnr 11/3728-1).

RESULTS

Female Acceptance of Foster Pups

The images from the game cameras—and subsequent investigations of the dens—showed that all four females moved their own pups and the foster pups to a new den site (Fig. 1). The time interval from when the staff left the enclosure, after the augmentation of the foster pups into the litter, until the female returned to the den ranged from four minutes to 950 min (16 hr 50 min). The time required for the females' to move the entire litter was estimated to be from 17 min to 30 min.

Growth Rates

At the augmentation, the foster pups were 4 days of age and had a body weight (BW 745 ± 35 g) corresponding to their biological siblings in the source litter (BW 710 ± 75 g). As expected their new stepsiblings in the recipient litter, at 12 days of age, were heavier (BW 1400 ± 122 g). The foster pups initially had a slower growth rate than their stepsiblings in the recipient litter as well as their biological siblings in the source litter. Twelve days after the augmentation, at 16 days of age, the foster pups had gained an average of 155 g (BW 900 ± 141 g) while their biological siblings had gained an average of 890 g (BW 1,600 ± 141 g) and their stepsiblings, at 24 days of age, had gained an average of 620 g (BW 2,020 ± 130 g). After another ten days, at 26 days of age, the foster pups had gained an average of 425 g (BW 1325 ± 35 g) while their biological siblings had gained an average of 1,100 g (BW 2,700 ± 283 g) and their stepsiblings, then at 34 days of age, had gained an average of 1,080 g (BW 3,100 ± 0 g) (Fig. 2).

The foster pups had a similar growth rate as their stepsiblings in the recipient litter, as well as their biological siblings in the source litter. At the time of augmentation, the foster pups were considerably heavier (BW 925 ± 35 g) than their biological siblings (BW 695 ± 74 g) and also heavier than the 3 days younger pups in the recipient litter (BW 825 ± 129 g). The foster pups were still slightly heavier (BW 3,050 ± 71 g) at 38 days of age, compared to their stepsiblings (BW 2,875 ± 194 g) at 35 days of age (Fig. 3).

Fig. 1. All of the females moved the entire litter to a new den site after the introduction of the foster pups. Shown here is the female at Polar Zoo.

Survival Rates

Overall, the survival until 33 weeks of age (0–231 days) for all pups born in 2011 was 65%. The survival was higher for foster pups (75%) than for pups that stayed with their biological mother (63%), but the difference was marginally significant ($\chi^2 = 0.06$, $P = 0.05$), nor were there any significant differences between foster pups and pups born in zoos between year 2000 and 2011 ($\chi^2 = 0.14$, $P = 0.704$). At Polar Zoo, the entire litter, both the foster pups and the female's own pups, died when the foster pups were between 6 and 37 days of age and the female's own pups were between 11 and 42 days of age (May 25th–June 21st). No traces of the pups were found.

DISCUSSION

The results show that female wolves initially accept foster pups added in her litter, that differences in growth rate increase with age differences between foster pups and the females' own pups, and that there is no significant difference in survival rate between foster pups and the females' own pups.

We augmented two foster pups in a litter that already consisted of eight pups because we did not have permission from the ethics committee to remove any pups other than intended foster pups. However, we recommend that the maximum size of a foster litter does not exceed the maximum litter size in the population. In the Scandinavian zoo population, the maximum litter size is nine pups.

All females in this study accepted the foster pups or failed to distinguish them. The pups' growth rates indicate that the likely affecting factor, competition over nipples, seems to depend on the age difference between the foster pups and the female's own pups. The survival rate to 33 weeks was even higher for foster pups than for pups that stayed with their biological mothers in the source and recipient litters.

Female Acceptance of Foster Pups

The success of cross-fostering may depend on the inability of the mother to recognize unfamiliar individuals in the litter. The females in this study moved all the pups, both foster pups and their own pups, to a new location in seemingly random order without discriminating between the pups. In the coyote study (Kitchen and Knowlton, 2005), it was shown that two of the three attempts with 3–4 weeks old pups succeeded, whereas none of the attempts to cross-foster 6–7 week-old

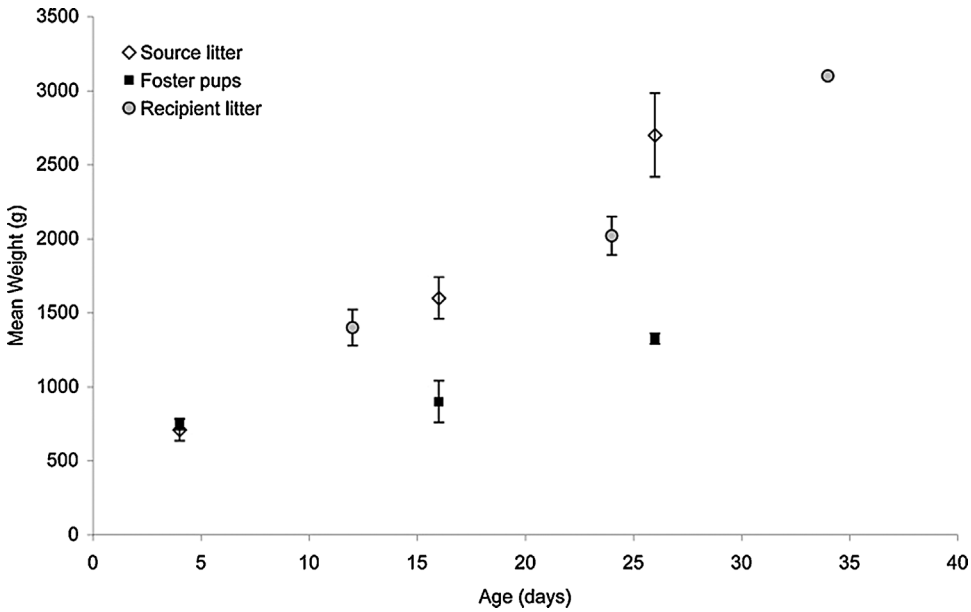

Fig. 2. Growth rate for the foster litter with −8 days age difference. Mean weight ± SD for foster pups, the female's own pups in the recipient litter and foster pups' biological siblings in the source litter.

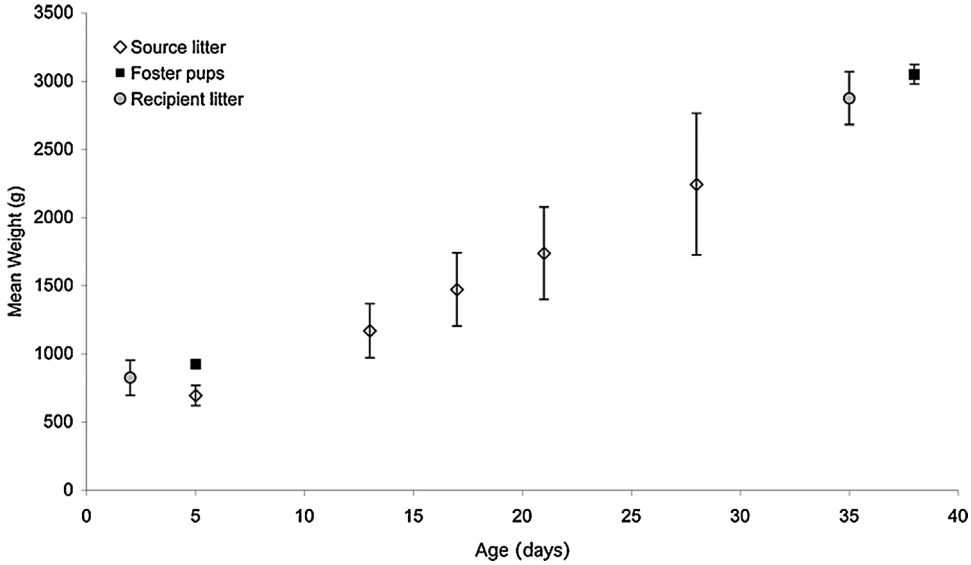

Fig. 3. Growth rate for the foster litter with +3 days age difference. Mean weight ± SD for foster pups, the female's own pups in the recipient litter and foster pups' biological siblings in the source litter.

pups succeeded. In the non-successful cases, the pups died within 24 hr (6–7 week-old pups) and 48 hr (3–4 week-old pups) of augmentation. This suggests there is a mechanism of kin recognition by parents, but that females probably do not learn to recognize their offspring until the pups are somewhere between 3–4 weeks and 6–7 weeks of age.

Growth Rates

The results show that 3 days older foster pups have essentially the same growth rate as their stepsiblings, while the foster pups that were 8 days younger had a much slower growth rate. The rate of growth depends on how well the pups are fed (Kreeger, 2003). In the litter with 3 days older foster pups, all pups were in approximately the same stage of development, and hence similarly tuned to suckling. In the litter with 8 days younger foster pups, the female's own pups had a major development advantage in the competition over the nipples: at the time of augmentation they were 12 days of age and had already started to open their eyes and were rather mobile. The foster pups were only 4 days of age, and were not only considerably smaller, but also blind and barely mobile. During this period, between the first and second weighing (at 4 and16 days of age), the foster pups gained an average of 155 g (17%). Wolf pups open their eyes at 12–14 days of age (Packard, 2003). The foster pups supposedly opened their eyes at this time and in the following period, between the second and third weighing (at 16 and 26 days of age), their growth rate improved and they gained an average of 425 g (32%). This increased weight gain hence may be due to an improved competitiveness as their eyes opened and they became more mobile. But still the foster pups could not catch up with their stepsiblings' head start in weight gain, and fell behind even more in comparison.

For cross-fostering to be successful, we recommend to match the age of the foster pups with the recipient litter as closely as possible, to ensure that all pups can compete on relatively equal terms.

Survival Rates

The survival rate for all pups was 65% during the first 33 weeks. The results show that foster pups have a slightly better survival than pups that stayed with their biological mothers. The results may serve as an indication of the foster pups' survival potential, although it must be emphasized that the sample size in the study is small and the causes of death probably have little to do with kin or non-kin related factors. The overall mortality was accordingly 35%. This is largely consistent with a study of wild wolves in North America (Fuller et al., 2003), which estimated pup mortality to 34% from early summer to late autumn/early winter.

Possibility to Implement Cross-Fostering in the Wild

In Scandinavia, wild females on average give birth from 19th of April until 20th of May, based on GPS data, (Nonaka, 2011), which is 2 weeks earlier than females in the zoos (Amundin, in press). The parturition period in the two populations is only likely to overlap by 1–2 weeks. In this case, only a limited number of zoo litters would be available for cross-fostering with wild wolves, as indicated by the foster pups' difficulties to compete over nipples with 8-days older pups in our study. In order to achieve genetic enhancement of a wild population, it is crucial that the cross-fostered pups survive and reproduce within the population. This is not to be taken for granted in Sweden

where there is a serious and polarized conflict between different interest groups about the wolf's presence in the country (Havula, 2006). There are a few forms of legal hunting that are sanctioned by political decisions, but there is also extensive illegal hunting amounting to half of the total mortality (Liberg et al., 2011). Potential cross-fostered pups must therefore be protected, not only while they stay in their foster parents' territory, but also during dispersal, which is common in the age range 1.1–1.3 years (Sand et al., 2010), and until they until they have reproduced and contributed genetically to the wild population.

CONCLUSIONS

The cross-fostering attempt between zoo litters has been successful. The foster pups coped with the competition over nipples, even with an age difference of up to 8 days. Therefore, the method can be recommended as a way to introduce potential new founder genes in the Scandinavian wild wolf population. The pups ought to be at least 4–6 days of age at the time of augmentation, and the age difference between foster pups and the female's own pups should probably not exceed 8 days, the largest age difference tested in this study.

If zoo pups are to be cross-fostered into wild wolf packs, their protection is crucial in order for them to reach adulthood and allow them to reproduce.

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