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Evidence of multiple breeding in a wolf pack in Galicia, Spain

Short title: Multiple breeding in a wolf pack in Spain

Abstract

Using camera traps, a non-invasive technique for wildlife monitoring, we have been obtaining information about grey wolf (*Canis lupus*) populations in Galicia in northwestern Spain, as part of long-term studies. Here, we describe the movements of a monitored pack comprising three adult wolves, one male and two females, located south-west of Santiago de Compostela. Videos and photos recorded in early July 2023 show the two females with large and distended nipple rows, moving one behind the other at a distance of three meters. In early August 2023, at the same camera trap site, six juvenile wolves were recorded moving together. Our observations support a case of a pack with two females, possibly mother and daughter, that bred with the same male, producing multiple litters. The higher number of pups in 2023 compared with the previous years' litters suggest that the females combined their pups in the summer. Although multiple breeding cases have been demonstrated in wolf packs in North America, Eastern Europe, and India, to our knowledge, our study represents the first case of the phenomenon in a Western European pack.

Key words: wolf, pack size, multiple breeding, camera trapping, Galicia, Iberian Peninsula.

Wolf packs usually have a single breeding pair. Peterson (1977) explains that mate preference and dominant hierarchy usually limit the ability of subordinate wolves to breed. He describes how mating most likely occurs between the dominant male and female in a pack, pair bonds between mates are very stable, and dominant wolves usually interfere with courtship attempts among subordinate wolves. Van Ballenberghe (1983) describes the wolf as a social, territorial carnivore whose packs in the wild generally produce only one litter per year, even when other sexually mature females are present.

Some studies largely support these claims. For example, in Isle Royale, Michigan, only one or two of about 12 females bred each year (Wolfe and Allen 1973), and in northeastern Minnesota, none of 20 packs studied produced more than a single litter per year (Packard and Mech 1980).

However, multiple breeding in wolves is known to occur occasionally. Evidence of this behaviour comes from the study of wolf packs in different regions, including North America (Minnesota: Mech and Nelson 1989; Idaho and Yellowstone National Park, Wyoming: Ausband 2018; Alaska: Murie 1944, Haber 1977, Van Ballenberghe 1983, Ballard et al. 1987, Meier et al. 1995), Eastern Europe (Belarus: Sidorovich and Rotenko 2019), and South Asia (India: Jadhav et al. 2005).

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Evidence of multiple breeding has largely been the result of field observations, radio-telemetry tracking, aerial monitoring, and genetic analysis. However, detecting multiple breeding is not always easy. Stenglein et al. (2011) reported that multiple litters typically remain undetected unless two den sites are observed, or genetic data are collected and analyzed. The authors emphasized that, without genetic information, they would have never suspected (or verified) the presence of two litters born in the Jureano Mountain pack in Idaho in 2008.

These types of reports add to our understanding of multiple breeding events, which is still limited. Therefore, reporting new cases is important to continue to develop deeper insight into this behaviour and the circumstances contributing to it. Here we present a case of multiple breeding in a wolf pack in Galicia, Spain.

Our study area consists of low-altitude mountains approximately 600 m above sea level. The area covers approximately 10,000 ha west of the city of Santiago de Compostela and east of the Atlantic coast from Ría de Muros and Noia (Figure 1).

The vegetation in the southern part of the study area is composed of large expanses of scrubland, mainly gorse (*Ulex europaeus*), as well as pine (*Pinus radiata*) plantations and isolated oak (*Quercus robur*) trees. In the central and northern parts, forest plantations (*P. pinaster*, *Eucalyptus globulus*, and *E. nitens*) predominate; corn is also cultivated here. Unlike the south, these areas are entirely rural, and do not have extensive livestock farming. However, there are some small livestock farms, mostly cattle in the north and cattle and sheep in the center. Disposal sites for cattle (mainly calves) and wild boar carcasses (generated from hunting drives), which may serve as potential sources of carrion, are also found in these regions. The human population lives in small, scattered villages. The population density in the south, center and north of the study area is 16.6, 15.8, and 31.1 people per km^2, respectively.

The first phase of wolf pack monitoring included walking surveys along dirt roads and firebreaks to locate wolf signs, mainly recent scats, that could indicate an active wolf route. From these surveys, we found signs of wolves in three separate zones within the study area, which we assumed corresponded to three packs. We then installed camera traps along these routes to confirm the presence of individuals and to monitor their movements. Through the recordings, we were able to identify some individuals by their characteristics. These identifications have been useful for recognizing individuals over time.

The cameras were placed on trees using a telescopic ladder, typically 3 to 4 m from the ground, at locations with the greatest number of wolf signs (scats). They were programmed to record 30-second videos or photos in bursts of three shots. Video mode was used for shorter recording durations (around two weeks), and photo mode, for longer periods (around one month). They were not programmed to record photos and video because we found that, by the time the video began recording, the animal was already out of view. A camera's tilt angle depended on the height at which the camera was placed, the distance from the tree to the dirt road, the road's width, and the terrain slope. The angle covering the dirt road depended on the trigger speed and

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recovery time of each camera model. In both cases, the angle ranged from 0 to 30 degrees.

Sampling was conducted using 26 camera traps deployed at 14 sites, for a total of 1522 sampling days, distributed among the three packs (Table 1, Figure 1). Cameras were occasionally replaced at some locations due to theft, malfunction, or performance improvements.

The first unequivocal evidence that the three groups were distinct packs was obtained during the 2022 breeding season, when reproduction was observed in all three groups. Furthermore, the camera recordings that year did not reveal the presence of individuals from one pack in the areas of the others during the breeding season.

Pack size is small, generally consisting of two to four individuals aged one year or older by the beginning of spring (Table 2). The mean pack size has been three individuals. This small number may be due to a high mortality rate in the study area. We have records of wolves killed in traffic accidents, juvenile wolves killed by livestock dogs, and sudden disappearances of wolves, possibly due to illegal hunting in some cases. We also have recordings of lame wolves in all three packs and suspect their injuries are the result of traffic accidents.

In the years monitored, there was only one male per pack, and all were of reproductive age. Interestingly, despite the small pack size, the reproduction rate was high (8 out of 10 possible reproductions). Most litters consisted of 1 to 4 pups, with one case of six pups (Table 2).

Here, we primarily focus on the data gathered on Pack 1, for which we have recordings of all the wolves in this pack from spring 2021 through fall 2023. The recordings of breeding females and pups confirmed reproduction occurred in Pack 1 during all three years of monitoring (2021–2023). Notably, the 2023 breeding season, during which only three adults were recorded, yielded a relatively high number of pups (n=6).

In a video recorded on July 3, 2023, we observed two females with distended and large nipple rows, signs they were weaning, moving one after the other less than three meters apart (Figure 2). On July 30, 2023, a pup was recorded at the same site were the two females were previously recorded together (Figure 3), and about a week later, on August 5, 2023, six pups were recorded there (Figure 4). We argue that these recordings provide evidence that multiple breeding occurred in this pack.

In accordance with the categorisation established by Barber-Meyer and Mech (2015) based on nipple size, the females in Pack 1 can be classified as ‘current breeders’. In addition to having large nipples, the field observations that we gathered (females moving near the denning area and the presence of pups) fits with this categorisation.

The relatively high number of pups in Pack 1 also strongly supports a multiple breeding event involving the two females and the single male. In previous years, the number of pups in Pack 1 was consistently low (from 1 to 3 pups). A plausible explanation for the

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number of pups in 2023 is that it reflects the combined breeding efforts of the two females.

Cases of pup pooling have been previously reported in the literature. Murie (1944) observed two litters of an Alaskan pack occupying the same main den in late June. Van Ballenberghe (1983) observed that, in mid-July, a litter of pups in a Minnesota pack was moved from a secondary den to the main den, where another litter was located. The dates when multiple litters were observed in the same den in these cases is consistent with our data from the Galician pack.

Video recordings of Pack 1 in 2023 show the presence of a single male who almost certainly mated with the two females. The Pack 1 male is lame, possibly the result of a traffic accident (Figure 5), making him easy to distinguish from the males in the other packs. Video recordings of this male show that he is well integrated in the pack and clearly plays the role of the dominant male. For example, his tail is raised when accompanying the females. The fact that no other solitary male was recorded rules out the possibility that paternity could lie with another individual.

Moreover, individuals from Packs 1 and 3 have never been recorded in each other's area; therefore, mating between Pack 1 females and the Pack 3 male was ruled out. Likewise, the Pack 2 male has never been recorded in Pack 1's area. In fact, as of November 2022, this male has not been in any recordings, suggesting he disappeared from the pack around that date and, thus, was not around during the 2023 breeding season to mate with any of the packs' females.

Our recordings indicate the lame dominant female of Pack 2 that bred in 2022 also was not present for the 2023 breeding season. Two females were recorded in Pack 2's area starting in March 2023; however, neither were the lame female. We assume that they were subadults born in 2022. The disappearance of both the dominant male and female from Pack 2, which bred continuously from 2019 to 2022, likely accounts for the lack of reproduction in the pack in 2023 (Table 2).

The absence of the dominant pair in Pack 2 may have been a factor influencing the movements of some Pack 1 individuals into Pack 2's area. Between November 4, 2022, around when the Pack 2 male putatively disappeared, and April 29, 2023, three individuals from Pack 1 travelled approximately 19 km into Pack 2's area at least 17 times. According to the recordings, the movements were spaced between 3 and 30 days apart. We could confirm they were from Pack 1 as an individual with very light, easily distinguishable fur from that pack participated in these movements. After this period, this light-coloured wolf no longer appears in the recordings. Curiously, the dominant, lame male from Pack 1 did not participate in any of these movements. From late April 2023 through the breeding period of that year, Pack 1 individuals were not recorded moving in Pack 2's area, possibly due to their focus on breeding tasks. We hypothesize that the movements were related to feeding activities, as we have a recording that shows wolves moving to feed on dead animals at a farm in Pack 2's area.

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Given that the Pack 2 male disappeared before the winter movements of Pack 1 individuals and, therefore, before the mating season, we can discard the possibility that he mated with the breeding females of Pack 1. Moreover, the Pack 1 male remained in his pack's area for nearly the duration of the 2023 breeding season, and likely beyond.

The relationship between the two breeding females of Pack 1 is unknown, but it could be a mother–daughter one, given their apparent tolerance and the small group size. Previous studies reference multiple breeding events in which the reproductive females were mother and daughter. Mech et al. (1998) and Mech (1999) propose that multiple breeding with closely related females may be reserved for dominant males in relatively small wolf packs consisting of multiple litters. Although Mech (1999) recognises that the genetic relationship of the mothers in these packs is unknown, he hypothesises that the mothers likely include the original matriarch and one or more of her daughters, and that the fathers are probably the patriarch and unrelated adoptees.

Based on the date and direction of the breeding females' movement, the scat evidence collected during walking surveys, and additional data on the 2024 breeding season provided by a collaborator (JR, pers. comm.), we estimated that the Pack 1 den or dens were located 1–2 km from the recording location that captured the videos of the breeding females and their pups. If there were two dens, we assume they were close to each other, as the two breeding females were moving together in the same direction in early July, during the weaning period (which is completed 9–10 weeks after birth; Packard et al. 1992), assuming parturition occurred in early May. These data contrast with those from the literature in which den distances corresponding to breeding females of the same pack ranged from 6 to 21 km (Murie 1944; Van Ballenberghe 1983; Mech and Nelson 1989).

In North America, the incidence of multiple breeding in wolf populations was estimated to be less than 15% (possibly less than 10%) (Mech 2024). Even so, this percentage still represents a significant number of cases, which could affect the population dynamics and demography in certain populations or regions. More data is needed to determine the prevalence of multiple breeding in European wolf populations, and the potential role the behaviour may play in their populations dynamics.

Multiple breeding in North American wolf populations is associated with large packs that feed on large prey and live at higher latitudes (Mech 2024). These traits do not characterize Pack 1 from Galicia, which have a small pack size (and a relatively low birth rate). The evidence gathered on this pack also indicate that prey consists of small- to medium-sized animals (e.g., wild boar, roe deer, rabbits). It also suggests that the local food supply may be scarce, as we observed these wolves periodically travelling long distances to scavenge dead livestock.

Our study represents the first described record of multiple breeding in a wolf pack in Western Europe. It also highlights camera traps as a highly effective technique for field studies of wolves. The factors involved in the ocurrence of multiple breeding in the

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Galician pack are unknown, but interestingly, likely involve circumstances that are different from those observed in the cases in North America. Therefore, it is crucial to report these cases and conduct more research on this behaviour in packs in Spain, and throughout Europe.

Acknowledgements

We recognise the great value of the work carried out by our colleague Agustín Pérez-Souto, who launched the Asociación Signatus. We thank the Dirección Xeral de Patrimonio Natural of the Xunta de Galicia for granting authorization to conduct the fieldwork. We also thank Eloi Rodríguez and José Rivas for sharing unpublished data from their personal field work, and Melinda Modrell for reviewing the English text. Finally, we thank the editors and the two anonymous reviewers for their suggestions, which greatly improved the manuscript.

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PACK NUMBER	Pack 1	Pack 2	Pack 3
NUMBER OF SIMULTANEOSLY ACTIVE CAMERAS	3	5	3
AVERAGE DISTANCE BETWEEN CAMERAS	173 m SE= 66,67	1413 m SE= 175,98	1004 m SE= 202,10
SAMPLING PERIOD	April 2021-October 2023	December 2019-January 2024	May 2022-December 2023
TOTAL NUMBER OF SAMPLING DAYS	944 days	1522 days	549 days
CAMERA ID AND MODEL	Location 1: EF13 Stealth Cam EF48 Coolife EF 50 Coolife EF67 Coolife. Location 2: EF05 Wimius EF40 Suntekcam EF86 Coolife Location 3: EF53 Apeman	Location 4: EF13 Stealth Cam EF17 Bolyguard EF49 Coolife Location 5: EF20 Victure EF26 Victure EF39 Coolife EF48 Coolife Location 6: EF22 Victure EF25 Apeman EF40 Suntekcam Location 7: EF26 Victure Location 8: EF51 Coolife Location 9: EF02 Victising EF41 Suntekcam EF67 Coolife EF87 Hapimp Location 10: EF34 Victure EF81 Coolife	Location 11: EF80 Coolife Location 12: EF65 Mini 301 Location 13: EF66 Mini 301 Location 14: EF401 Suntekcam EF482 Suntekcam

PACK NUMBER AND YEAR	Nº OF PUPS	Nº OF FEMALES 1 YEAR OLD OR OLDER	Nº OF MALES 1 YEAR OLD OR OLDER
Pack 1 2021	3	2 or 3	1
Pack 1 2022	1 or 2	2 or 3	1
Pack 1 2023	6	2	1
Pack 2 2019	4	2	1
Pack 2 2020	3	2	1
Pack 2 2021	4	2	1
Pack 2 2022	4	2	1
Pack 2 2023	0	2	0
Pack 3 2022	4	1	1
Pack 3 2023	*No reproduction	N.D.	N.D.

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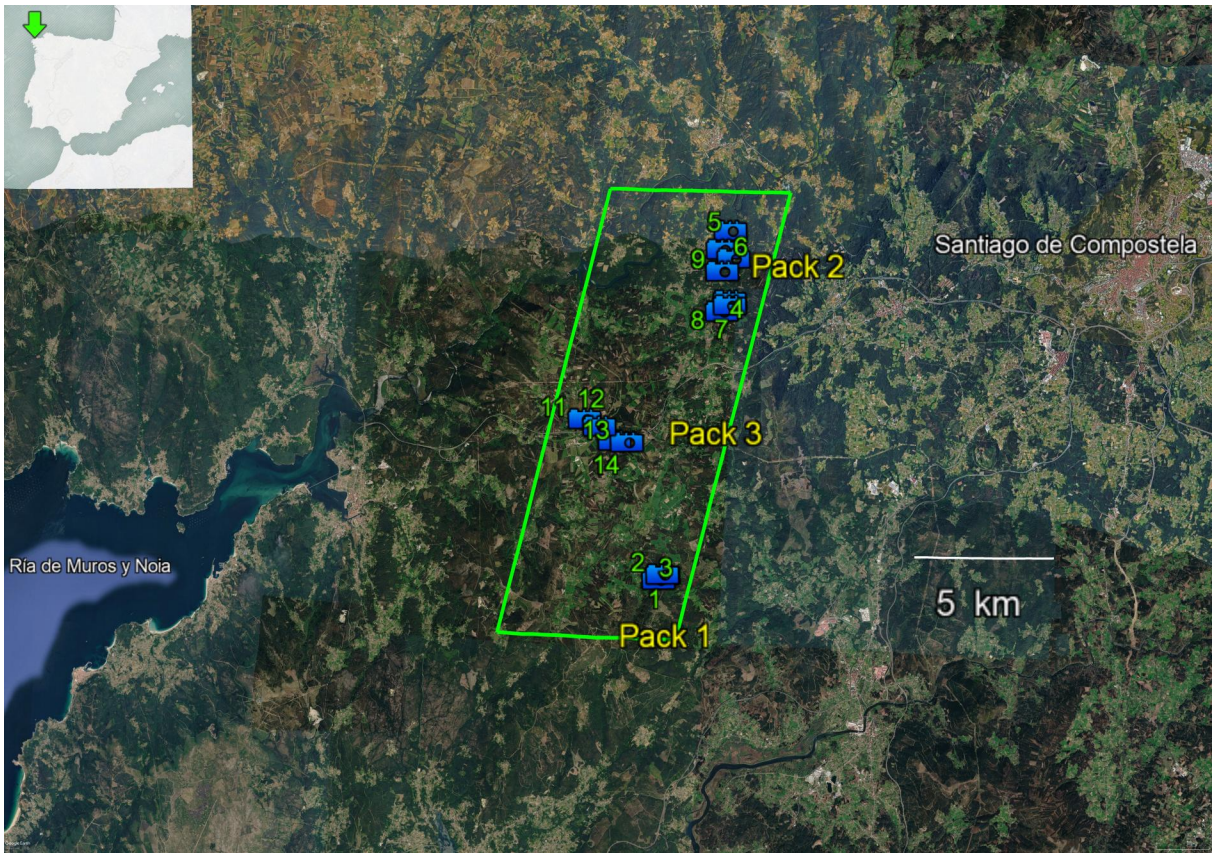


Figure 1. Study area and camera locations in each pack.

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Figure 2. Frame from a video showing the two breeding females from Pack 1 moving together in close proximity. (Video recorded on July 3, 2023).

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Figure 3. Frame from a video showing the presence of one pup. (Video recorded on July 30, 2023).


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Figure 5. Frame from a video showing the Pack 1 male. Note his right hind leg is displaced inwards and twisted, which causes him to have a discernable limp. This feature facilitated his identification in the recordings. (Video recorded on August 16, 2023).

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Tables

Table 1 - Download source file (14.64 kB)
Table 1. Information on camera-based sampling. SE= Standard error.

Table 2 - Download source file (12.4 kB)
Table 2. Annual composition of wolf packs. The number of females and males that are at least 1-year-old and the number of pups per pack by observation year are provided. (*) Unpublished data from a collaborator (JR) indicates that there was no reproduction in this pack in 2023. (N.D.) Not determined.

Figures

Figure 1 - Download source file (22.13 MB)
Figure 1. Study area and camera locations in each pack.

Figure 2 - Download source file (272.58 kB)
Figure 2. Frame from a video showing the two breeding females from Pack 1 moving together in close proximity. (Video recorded on July 3, 2023).

Figure 3 - Download source file (799.53 kB)
Figure 3. Frame from a video showing the presence of one pup. (Video recorded on July 30, 2023).

Figure 4 - Download source file (267.5 kB)
Figure 4. Frames from a video showing the presence of six pups, numbered according to the order of their appearance in the video. (Video recorded on August 5, 2023).

Figure 5 - Download source file (75.39 kB)
Figure 5. Frame from a video showing the Pack 1 male. Note his right hind leg is displaced inwards and twisted, which causes him to have a discernable limp. This feature facilitated his identification in the recordings. (Video recorded on August 16, 2023).

Evidence of multiple breeding in a wolf pack in Galicia, Spain

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A - Research concept and design, B - Collection and/or assembly of data, C - Data analysis and interpretation, D - Writing the article, E - Critical revision of the article, F - Final approval of the article

Abstract:
Using camera traps, a non-invasive technique for wildlife monitoring, we have been obtaining information about grey wolf (*Canis lupus*) populations in Galicia in northwestern Spain, as part of long-term studies. Here, we describe the movements of a monitored pack comprising three adult wolves, one male and two females, located south-west of Santiago de Compostela. Videos and photos recorded in early July 2023 show the two females with large and distended nipple rows, moving one behind the other at a distance of three meters. In early August 2023, at the same camera trap site, six juvenile wolves were recorded moving together. Our observations support a case of a pack with two females, possibly mother and daughter, that bred with the same male, producing multiple litters. The higher number of pups in 2023 compared with the previous years' litters suggest that the females combined their pups in the summer. Although multiple breeding cases have been demonstrated in wolf packs in North America, Eastern Europe, and India, to our knowledge, our study represents the first case of the phenomenon in a Western European pack.

Keywords: wolf, pack size, Iberian Peninsula, Galicia, multiple breeding, camera trappping.

Received: 2026-05-11
Revised: 2026-11-03
Accepted: 2026-11-05
Final review: 2026-09-21

Short title
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