

THE MONOPHYLETIC ORIGIN OF DELAYED IMPLANTATION IN CARNIVORES AND ITS IMPLICATIONS

PATRIK LINDENFORS,¹ LOVE DALÈN,² AND ANDERS ANGERBJÖRN³
Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden

¹*Patrik.Lindenfors@zoologi.su.se*

²*Love.Dalen@zoologi.su.se*

³*Anders.Angerbjorn@zoologi.su.se*

Abstract.—In several carnivores a newly fertilized egg enters diapause instead of being directly implanted into the uterus, a phenomenon called delayed implantation. Several hypotheses have been forwarded to explain the utility of this prolonged gestation period, but all of these depend on several independent origins of the character. Here, we conduct a phylogenetic reconstruction of the evolution of delayed implantation in the Carnivora that reveals one basal origin, with additional transitions all having occurred within the Mustelidae. Hence, previous hypotheses relating to its evolution become untestable. Further analyses revealed that the presence or absence of delayed implantation is unrelated to the timing of mating season and birth season. Instead, mustelids with direct implantation are smaller than those with delayed implantation. We therefore suggest that delayed implantation has been selected against in small species due to the relatively higher fecundity costs of a prolonged gestation period.

Key words.—Mustelids, phylogenetic comparative methods.

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The phenomenon of delayed implantation, in which a newly fertilized egg enters diapause instead of being directly implanted into the uterus, occurs in a number of caniform carnivores. This prolongs the period between mating and parturition resulting in a longer total gestation period. After the delay, implantation in these carnivores occurs seasonally, meaning that the implantation time of the blastocyst occurs more or less simultaneously in all females of a population (Mead 1989; Sandell 1990). The timing of this implantation is generally determined by photoperiod changes (Mead 1989), but for example, in the European badger (*Meles meles*) the timing of implantation can be modified by up to 37 days pending the female's nutritional state (Ferguson et al. 1996).

The utility of delaying implantation has puzzled a number of researchers, provoking a number of hypotheses to why it has evolved (for reviews see Mead 1989; Sandell 1990). Such a hypothesis must explain why both mating season and parturition date are optimally placed at a certain time of the year. An early proposition was that the time of mating was determined by the animals' nutritional status and hence fixed to a certain time of the year. But because giving birth after a "normal" gestation period would render the rearing of the young impossible, and thus be detrimental to the survival of the species, delayed implantation evolved (Fries 1880).

A later suggestion turned the reasoning around and argued that parturition date is fixed because offspring need the maximum possible time to get ready for overwintering. This timing of the parturition would, however, require the mating season to occur in the winter when low temperatures, it was argued, are detrimental to both sperm production and the possibility of males and females to meet (Prell 1930). Another suggestion at the time was that delayed implantation is a character without any adaptive value (Hamlett 1935).

Heidt (1970) later argued that delayed implantation in small mustelids has evolved as a mechanism to limit popu-

lation size. This would be accomplished as the relatively longer total gestation period reduces the number of litters per year. As this hypothesis depends on a form of group selection that has since been shown not to stand up to closer scrutiny (e.g., Williams 1966), it is of little interest. It nevertheless highlights the central problem surrounding the evolution of delayed implantation: of what utility is a possible reduction in the number of litters per year?

Currently, one consensus hypothesis states that delayed implantation in carnivores has evolved for mating when animals are in prime condition and for parturition when resources are at a maximum (Mead 1989; Sandell 1990 and references cited therein), a proposition not very different from the very first (Fries 1880). Sandell (1990) has provided a theoretical framework for how mating and parturition dates can be selected away from each other. In his proposal, it is assumed that female choice is facilitated by an early mating season, as males should be more able to compete when the conditions are favorable. Alternatively, in species that aggregate once a year (e.g., many—but not all—pinnipeds), the optimal mating season should be during this aggregation period, or, in species that hibernate during winter (e.g., many—but not all—ursids), delayed implantation could be an adaptation to the time constraints of winter sleep.

The ability to test all the hypotheses formulated to date relies heavily on the assumption that delayed implantation has evolved separately several times in the carnivores. For example, Mead (1989) has claimed that the spotty distribution of species displaying delayed implantation within the carnivores strongly suggests that delayed implantation has several independent origins. According to Sandell (1990), delayed implantation has at least evolved independently 12 times in the carnivores. In this paper we test this assertion of multiple origins by reconstructing the most parsimonious evolution of delayed implantation in several alternative phylogenetic hypotheses of the carnivores.

Because our results show a probable monophyletic origin of delayed implantation (see below), we suggest that testable hypotheses should deal with the loss of delayed implantation as opposed to the traditional hypotheses that deal with its origin. Further, if adaptive hypotheses of origins or losses of delayed implantation are to be tested, our results point to the Mustelidae as the only group with sufficient variation in this character.

Another assumption by previous hypotheses is that species with delayed implantation have more fixed mating and parturition seasons. If a species has no season to adapt to, there is scarce reason to reproduce at a specific time of the year. Why delay reproduction more than necessary for energetical purposes? There are recognized exceptions to this assumption, such as the sea otters *Enhydra lutris* who have delayed implantation but no specific parturition season (Mead 1989), but in general the pattern is assumed true. Here we test this assumption by comparing the length of the mating and birth seasons between species with and without delayed implantation.

To take the analysis one step further, we analyzed what differences exist between those mustelids that have delayed implantation and those that do not. The question then becomes for what species a longer gestation period not only is of no utility, but possibly even detrimental. This can, for example, concern species that have so short life spans that they have no time to wait, species with unpredictable mortality causes so that delaying any aspect of reproduction incurs high probabilities of failed life-time procreation, or species in which several litters a year is a realizable possibility. Because small body size normally unites species with these characteristics (e.g. Harvey et al. 1989; Roff 1992; Stearns 1992; Charnov 1993), we tested the hypothesis that species lacking delayed implantation are smaller than their sister species having delayed implantation.

MATERIALS AND METHODS

Data for the absence or presence of delayed implantation in carnivores were taken from Mead (1989), complemented with data on *Lontra longicaudis* (has delayed implantation: Larivière 1999). Body weights were taken from Bininda-Emonds (1998) and Nowak (1997), complemented with data from the series "Mammalian Species" (Estes 1980; Powell 1981; Clark et al. 1987; Youngman 1990; Kinlaw 1995; Pasitschniak-Arts and Larivière 1995; Sheffield and Thomas 1997; Larivière and Walton 1998; Larivière 1999; 2001a; 2001b; Verts et al. 2001). Data on the length of the breeding season were taken from Mead (1989). All continuous variables were log-transformed prior to analysis. The data are available from the authors on request.

A search for life-history characters (mainly interbirth intervals, birth rates, and longevity) of the mustelid species in question was made, but as relevant data of good quality are scarce, the results of these analyses are not presented. Instead directions for future studies are discussed below.

We mainly base our conclusions in this paper on a complete phylogeny of the extant carnivores made with a super-tree technique combining a large number of source phylogenies, both molecular and morphological, by Bininda-Emonds et al.

(1999). These results were compared to those obtained from using two alternative phylogenies based on morphological data (Tedford 1976; Wozencraft 1989).

The hypotheses of evolutionary history of delayed implantation were reconstructed using the unordered parsimony algorithm in MacClade (Maddison and Maddison 2000). To test if species lacking delayed implantation had more fixed (shorter) mating and breeding seasons, and also if they are smaller than their sister species, we used matched pairs comparisons (Møller and Birkhead 1992; Wickman 1992) (Appendix).

RESULTS

Out of the 69 carnivore species for which information is available, 46 were reported as having delayed implantation whereas 23 were reported as not having delayed implantation. A further six carnivore families are reported not to contain species having delayed implantation (Mead 1989). These were included in the analysis on a family level.

The reconstruction of ancestral states on the phylogeny of Bininda-Emonds et al. (1999) reveals a total of 13 gains and/or losses of delayed implantation in caniform carnivores. At one extreme, delayed implantation has evolved once in the basal part of the phylogeny and then been lost a maximum of 12 times. Alternatively, the other extreme solution is that after the initial basal gain of the character, delayed implantation has been lost once and regained 11 times. All alternatives between these extremes are equally parsimonious. However, and this is a crucial point, all of the potential regains of delayed implantation fall within the Mustelidae (Fig. 1).

For both the other alternative phylogenies used, 13 gains and losses were reconstructed. This is the same number of changes as reconstructed when using Bininda-Emonds et al.'s (1999) phylogeny. As before, one parsimonious solution for Tedford's (1976) phylogeny is a single basal gain of delayed implantation. Alternatively, however, an equally parsimonious solution is that delayed implantation has two basal origins. Once at the base of an Ursidae-Ailurus-Otaridae-Odobenus clade and once at the base of a Phocidae-Mustelidae clade. As was the case when using the Bininda-Emonds et al.'s (1999) phylogeny, however, all the other potential gains fall within the Mustelidae.

Wozencraft's (1989) phylogeny confirms the possibility of Tedford's (1976) phylogeny, again reconstructing two basal origins. One at the base of an Ursidae-Ailurus-Otaridae-Odobenus clade and one at the base of a Phocidae-Mustelidae clade. For this phylogeny, however, the possibility also exists that delayed implantation evolved separately for the Phocidae and the Mustelidae. As was the case for the other two phylogenies, however, the majority of changes fall within the Mustelidae clade. The bi- and triphyletic basal origins of delayed implantation on Wozencraft's (1989) and Tedford's (1976) phylogenies rely heavily on the paraphyletic status of the pinnipeds, however, a notion that has become questionable with the advent of molecular carnivore phylogenies (e.g., Lento et al. 1995).

Mustelid species with delayed implantation did not have a shorter mating season (Wilcoxon's matched pairs $P = 0.878$, $n = 10$). Neither did they have a more fixed birth

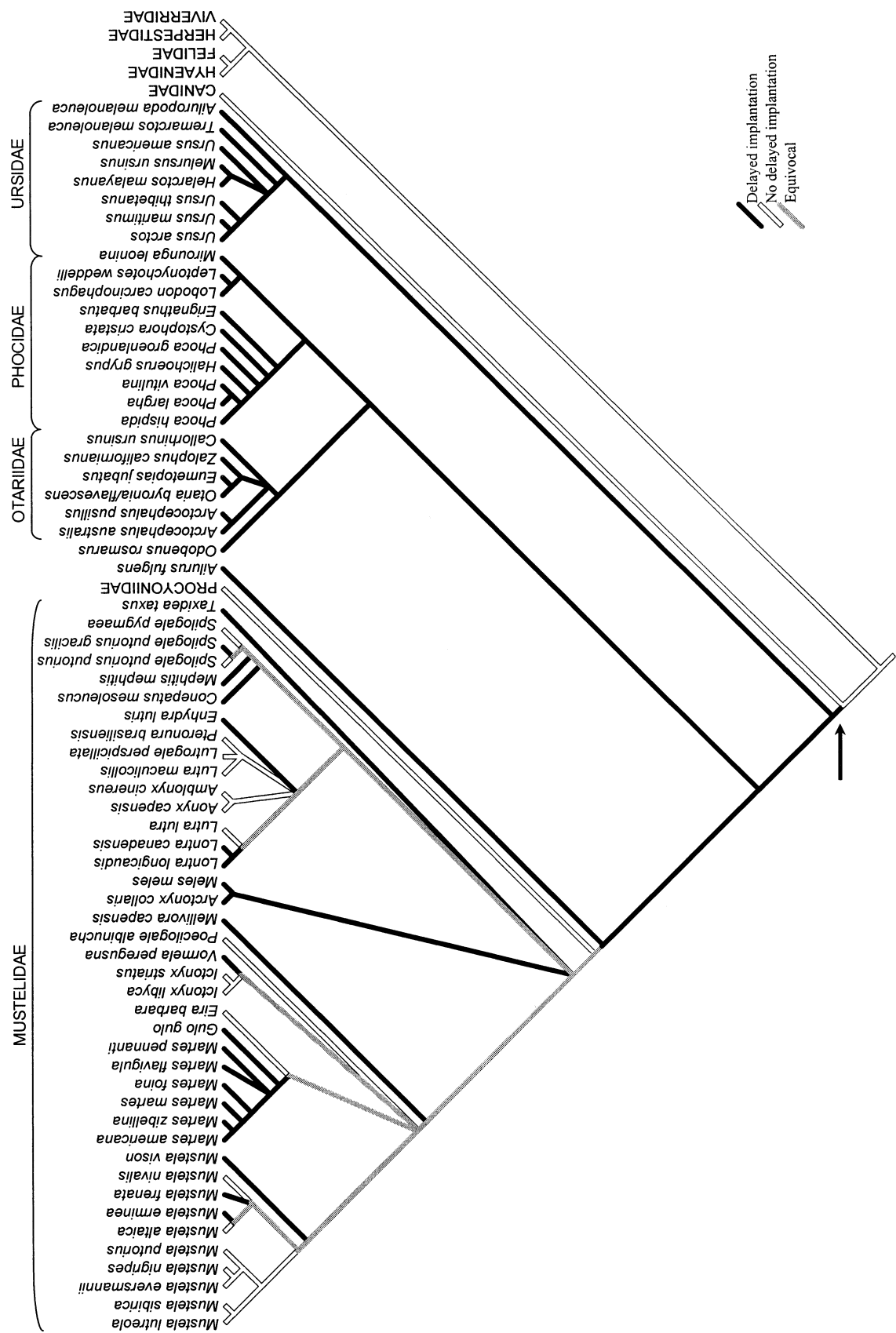


FIG. 1. Phylogeny showing the most parsimonious reconstruction of the evolution of delayed implantation in the carnivores. Note that the mustelids are numerically over-represented. The most parsimonious reconstruction of ancestral states on the carnivore super-tree revealed a total of 13 gains and/or losses of delayed implantation. At one extreme, delayed implantation evolved once in the basal part of the phylogeny (arrow) and has then been lost 12 times. Alternatively, the other extreme solution is that after the initial basal gain of the character, delayed implantation has been lost once and regained 11 times. All alternatives between these extremes are equally parsimonious. However, all of the potential losses or regains of delayed implantation fall within the Mustelidae.

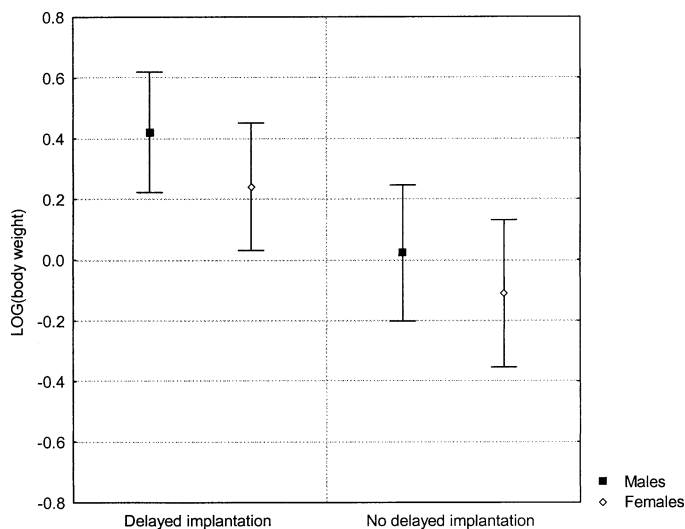


FIG. 2. Diagram illustrating the difference in body weight between mustelid species having delayed implantation and their sister species, or sister groups. The difference in size is significant whether the weights of males (squares), females (diamonds), or species averages (not shown) are analyzed.

season ($P = 0.169$, $n = 10$). Thus, it seems that seasonality cannot explain the absence/presence of delayed implantation in carnivores. Possible reasons for this are discussed below.

Mustelid species with direct implantation were, however, significantly smaller than their most closely related sister-species having delayed implantation. This was true for females (Wilcoxon's matched pairs $P = 0.037$, $n = 10$), males ($P = 0.028$, $n = 10$), and for average species size ($P = 0.016$, $n = 11$) (Fig. 2).

DISCUSSION

The results presented here strongly suggest a basal monophyletic origin of delayed implantation in carnivores (Fig. 1). Possible later independent origins all fall within the mustelid clade, thus limiting the possibility of testing a number of previous hypotheses (Mead 1989; Sandell 1990).

Circumstantial evidence indicates that the events in the mustelid clade are losses, not gains, of delayed implantation. For example, no physiological differences in how delayed implantation functions have been discovered, though only a few species have been fully investigated thus far (Mead 1989). Also, the origin of such a complicated physiological character as delayed implantation is much less probable than its loss. Furthermore, fertilized eggs of the domestic ferret, *Mustela putorius*, a species without delayed implantation can withstand brief periods of experimentally induced diapause (Foresman and Mead 1978). More detailed knowledge of phylogenetic relationships and distribution of delayed implantation within the mustelids will shed light on this question.

Our results also indicate that the presence or absence of delayed implantation does not coincide with a shorter duration of neither mating or birth season. Thus, the exact timing of these events seems no more important to species with delayed implantation as compared to those with direct im-

plantation. This result is somewhat unexpected given that species with delayed implantation generally are assumed to be living in more seasonally variable environments. Ferguson et al. (1996) have even validated this pattern statistically, although their test was limited to North American carnivores.

If we assume, however, that delayed implantation in the carnivores has a pure monophyletic origin with no additional gains in the phylogeny, the lack of significant differences on mating and birth season duration makes more sense. In this case, delayed implantation is a character lost when fecundity costs become too large, and not necessarily when the timing of mating and birth season has become comparatively less important. This is expected to happen in species with unpredictable mortality, species with a potential for more than one litter per year, or species with brief lifespans. All these characters normally correlate with small body size (e.g., Harvey et al. 1989; Roff 1992; Stearns 1992; Charnov 1993).

Because the mustelids are the group containing all instances of losses or regains of delayed implantation, we could test for the predicted size differences in this group only. Our analyses could identify the crucial difference between species with and without delayed implantation; the latter are significantly smaller than the former (Fig. 2). Such size differences are normally correlated with differences in a number of life-history characters such as age at maturity, birth rate, and longevity—all affecting life-time fecundity (e.g., Harvey et al. 1989; Roff 1992; Stearns 1992; Charnov 1993). Prolonged gestation time can thus be a factor selected against by fecundity selection. A suggestion for future research would hence be to focus on life-history differences other than gestation time between species with and without delayed implantation. Data exact enough for such an investigation are not available for enough species at the moment, however.

Given that this result rests on the phylogenetic hypothesis underlying the comparative tests, it is important to point out that the phylogeny of Bininda-Emonds et al. (1999) is a type of consensus tree utilizing all phylogenetic information published up until its construction. It thus unites knowledge gathered from both molecular and morphological phylogenies. This does not mean that it is the final truth, only that it is supported by a larger set of information than any alternative phylogeny.

Seasonal delay of implantation is known to occur outside the carnivores in one or several species belonging to five other orders of mammals: Diprotodontia, Edentata, Chiroptera, Artiodactyla, and Insectivora. For future hypotheses and analyses of possible processes responsible for the evolution of delayed implantation, and not only its loss, these other occurrences have to be considered simultaneously.

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APPENDIX

Matched Pairs Comparisons of Sister Taxa	
With delayed implantation	Without delayed implantation
<i>Mustela erminea</i>	<i>Mustela altaica</i>
<i>Mustela frenata</i>	<i>Mustela nivalis</i>
<i>Mustela vison</i>	<i>Mustela sibirica</i> , <i>M. eversmannii</i> , <i>M. nigripes</i> , <i>M. putorius</i>
<i>Martes americana</i> , <i>M. zibellina</i> , <i>M. martes</i> , <i>M. foina</i> , <i>M. flavigula</i> , <i>M. pennanti</i> , <i>Gulo gulo</i>	<i>Eira barbara</i>
<i>Vormela peregusna</i>	<i>Ictonyx libyca</i> , <i>I. striatus</i>
<i>Mellivora capensis</i>	<i>Poecilogale albinucha</i>
<i>Lontra longicaudis</i> , <i>L. canadensis</i>	<i>Lutra lutra</i>
<i>Enhydra lutris</i>	<i>Aonyx capensis</i> , <i>Amblonyx cinereus</i> , <i>Lutra maculicollis</i> , <i>Lutrogale perspicillata</i> , <i>Pteronura brasiliensis</i>
<i>Spilogale putorius gracilis</i>	<i>Spilogale putorius putorius</i>
<i>Mephitis mephitis</i>	<i>Spilogale pygmaea</i>
<i>Arctonyx collaris</i> , <i>Meles meles</i> , <i>Taxidea taxus</i>	<i>Conepatus mesoleucus</i>