

## THE EVOLUTION AND MAINTENANCE OF DELAYED IMPLANTATION IN THE MUSTELIDAE (MAMMALIA: CARNIVORA)

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**Abstract.**—Diapause, the temporary cessation of development at an early life-history stage, is widespread among animals and plants. The range of taxa exhibiting various forms of diapause indicates its enormous ecological significance and highlights its value as a model for examining life-history trait evolution. However, despite the impact of diapause on species ecology, there is little understanding of its adaptive value in many groups. Furthermore, the relative roles of phylogeny and ecology in determining the contemporary expression of the trait remain unresolved. Delayed implantation (DI) is a type of diapause found in several orders of mammals. It is particularly prevalent in the Mustelidae, with mustelids making up more than half of all mammals known to exhibit DI. This taxon is thus ideal for examining life-history predictors of DI and investigating the mode of evolution. Both maximum likelihood and maximum parsimony methods of ancestral state reconstruction indicated DI to be plesiomorphic in the mustelids, although multiple state changes are required to explain its contemporary distribution. After controlling for phylogeny, species with and without DI could be discriminated using just three variables: longevity, maximum latitude of the geographical distribution, and a term describing maternal investment. Our analyses supported the hypothesis that DI is more prevalent in seasonal climates. We also showed that longer-lived species are more likely to exhibit DI, suggesting a time cost to the trait. We found no correlate for the highly variable duration of DI, which remains unexplained. Although ecological factors can predict the distribution of DI in modern mustelids, phylogenetic constraint is likely to play an important role.

**Key words.**—Ancestral state reconstruction, diapause, generalized least squares, life-history evolution, phylogeny.

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Trait distribution among contemporary species is determined by a combination of phylogenetic and ecological factors. Although the role of phylogeny in the expression of ecological traits is unresolved (Freckleton et al. 2002), life-history traits in general appear to be relatively labile, exhibiting comparatively little phylogenetic constraint and being heavily influenced by prevailing environmental variables (e.g., home range size: Gittleman and Harvey 1982; monogamy: Komers and Brotherton 1997; maternal care: Fisher et al. 2002). This arises because the optimal states of such traits are often different under different environmental conditions, and these conditions in turn usually vary in space and time. As a result, it is common for life-history traits to be either gained or lost multiple times within taxa (e.g., aseasonal breeding in dasyurid marsupials: Krajewski et al. 2000; infant carrying in primates: Ross 2001; live bearing in fishes: Goodwin et al. 2002; transition to non-feeding larval stage in echinoderms: Villinski et al. 2002). Thus, analyses of the development of life-history strategies rely on an understanding of both phylogenetic constraints and the ecological variables that influence their expression.

Embryonic diapause, the temporary cessation of embryonic development, is a very widespread, yet poorly understood, life-history strategy in birds, fish, insects, plants, and mammals (where it is usually termed “delayed implantation”; Mead 1993; Renfree and Shaw 2000). Notably, diapause occurs patchily within taxa, being more abundant in some families than others (e.g., within families of insectivores: Vogel 1981; crustaceans: Fryer 1996; flowering plants: Forbis et al.

2002). The great diversity of groups exhibiting diapause suggests a considerable selective advantage (Renfree and Shaw 2000), and its patchy distribution in evolutionary history implies a strong phylogenetic component and multiple acquisitions. The nature of the ecological benefit imparted by diapause is unresolved for most taxa, and still less is known about the evolutionary constraints on contemporary distribution. Here we examine various hypotheses for the evolution of delayed implantation among one group of mammals, the family Mustelidae.

Our interest in the mustelids as a model for the evolution of delayed implantation (DI) lies first in the extraordinary prevalence of this trait in the family. While obligate DI has been reported in at least 53 mammal species, seven orders, and 10 families (Sandell 1990), almost half of these species are in just one family, the Mustelidae (Renfree and Shaw 2000). About 34% of mustelids have DI (Mead 1989; Sandell 1990), compared to fewer than 0.05% of mammals overall. Second, although the trait is thought to have arisen independently several times in the Mammalia (Mead 1993), the pattern of trait evolution has not explicitly been tested.

The mustelid family exhibits patchy expression of the trait, a number of mustelid subfamilies and even genera include some species with DI and others without (Aitken 1977; Mead 1989, 1993; Sandell 1990). Particularly striking are the pairs of closely related, and often ecologically similar, species, in which one exhibits DI and the other does not (e.g., Mead 1981; King 1984; Sandell 1984). Among these are the North American river otter (*Lontra canadensis*, with DI) and European river otter (*Lutra lutra*, no DI); the stoat (*Mustela erminea*, DI) and weasel (*M. nivalis*, no DI); and the western (*Spilogale gracilis*, DI) and eastern (*S. putorius*, no DI) spot-

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ted skunk. The mustelids' morphological diversity, widespread distribution, variation in life-history patterns, and irregular, abundant expression of DI make them ideal for examining hypotheses regarding the evolution of this trait.

As yet, the selective advantage offered by DI has not been identified with certainty. However, the most widely accepted explanation for DI is that it allows mating and parturition to be temporally uncoupled. As a consequence, animals are largely freed from the potentially conflicting pressures of selecting mating and parturition times that coincide with external optima, and each can take place at independently favorable times of year (Fries 1880; Aitken 1977; Mead 1989, 1993; Sandell 1990; Renfree and Shaw 2000).

The precise value of this uncoupling mechanism remains uncertain. Early hypotheses focused on the possibility that the uncoupling effect of DI would enable the young to be born earlier in the growing season, giving them the maximum possible time to develop before their first winter (Fries 1880; Prell 1930). The parturition timing hypothesis has more recently been supported by studies indicating that species with delayed implantation tend to give birth earlier than those without (Mead 1968, 1989; Sandell 1990; Ferguson et al. 1996). This probably arises because the photoperiod trigger that stimulates implantation also initiates the mating season, and thus the delayed blastocysts implant at the same time as mating begins in species with direct development (Mead 1989). Furthermore, if the ultimate benefit of temporal uncoupling lies in the timing of parturition, the effect should be most pronounced in seasonal environments (Conaway 1971; Vogel 1981; Sandell 1984). This appears to be the case for North American carnivores (Ferguson et al. 1996), and in some groups DI is more common at higher latitudes than near the equator (e.g., bats: Racey 1982; Bernard and Cummings 1997).

In contrast, Sandell (1990) argued that because parturition timing is a critical factor determining offspring survival, it should be under strong selective pressure even in the absence of DI. Sandell therefore proposed that DI would allow mating to move to the optimum time, perhaps when females have the greatest opportunity to select high-quality mates. Birkhead and Møller (1993) suggested that this sexual selection hypothesis may apply to all temporal uncoupling mechanisms, namely sperm storage, delayed development, and delayed implantation. This highlights the possibility that the adaptive value of embryonic diapause may be linked to that of sperm storage and delayed development, so that identifying the benefits of one may lead to a better understanding of the others. Finally, it should be borne in mind that DI allows the timing of both parturition and mating to be simultaneously optimized, and there may not always be conflict between the two.

Using the Mustelidae to examine the evolution of DI, we addressed the following questions and hypotheses. What is the historical status of DI among the Mustelidae? Is the contemporary distribution of DI best explained by shared inheritance from the common ancestor of modern mustelids and selective evolution of direct development or by multiple acquisitions of the trait? The ancestral state of the trait influences the likely ecological and evolutionary processes leading to its present distribution; selective evolution of di-

rect development emphasizes a cost to DI, while multiple acquisitions imply strong positive selection. This has broader relevance to theories of life-history evolution, where there is ongoing debate about the lability of life-history traits (Freckleton et al. 2002). Furthermore, a high degree of lability may reduce the significance of phylogenetic constraint in character evolution, leading us to the second question: What is the role of phylogeny in the expression of this trait among modern mustelids? The degree of phylogenetic constraint on DI distribution influences the relative importance of ecological correlates. Of the ecological variables under consideration, we predicted that DI would be more common among species closer to the poles than those with an equatorial distribution (Racey 1982; Bernard and Cummings 1997). This is expected if DI acts as an uncoupling mechanism to allow mating and parturition to occur in optimal seasons; because seasonality is reduced at the equator, the timing of these events might be less constrained by a rigid link between mating and parturition. As an extension, we predicted that the delay would be longer in species further from the equator, in response to the increasingly longer winters. Because DI effectively increases the minimum time between reproductive events, we tested the hypothesis that the trait is more prevalent in longer-lived species, which are better able to spread reproductive investment over several years. As a result, we expected that females might increase per-litter investment, to minimize the risk of reproductive failure.

## MATERIALS AND METHODS

### *Reconstruction of Ancestral Character States*

Two methods, maximum parsimony (MP) and maximum likelihood (ML), are commonly used to map the evolution of characters on known phylogenies. MP methods select the state of each ancestor in the configurations that minimize the number of character state transitions over the tree (Cunningham et al. 1998; Maddison and Maddison 2001). MP methods have some limitations: for instance, because MP takes no account of branch lengths, change on long branches may be underestimated (Pagel 1999a). Furthermore, MP may be unreliable where probabilities of gains and losses of a trait are unequal (Cunningham et al. 1998), or where character trait transition is frequent (Frumhoff and Reeve 1994; Schultz et al. 1996). Finally, parsimony methods cannot measure probabilistically the uncertainty that is inherent in ancestral state reconstruction.

By contrast, ML methods consider specific models of evolution and select the model that best explains the observed data (Pagel 1999b). The relative probability of each character state at each node is considered independently, meaning that less parsimonious reconstructions can result (Cunningham et al. 1998). The rates of transition from  $0 \rightarrow 1$  ( $\alpha$ ) and  $1 \rightarrow 0$  ( $\beta$ ) are fitted by ML and can be independent (so that  $\alpha \neq \beta$ ), or equal ( $\alpha = \beta$ ). The effect of branch length on the likelihood of a character changing state can be examined by scaling branch lengths according to the parameter  $\kappa$ , (Pagel 1999a, b). Values of  $\kappa < 1$  indicate that traits evolve rapidly before stabilizing (with the extreme of  $\kappa = 0$  implying a punctuational model of evolution).  $\kappa > 1$  suggests traits

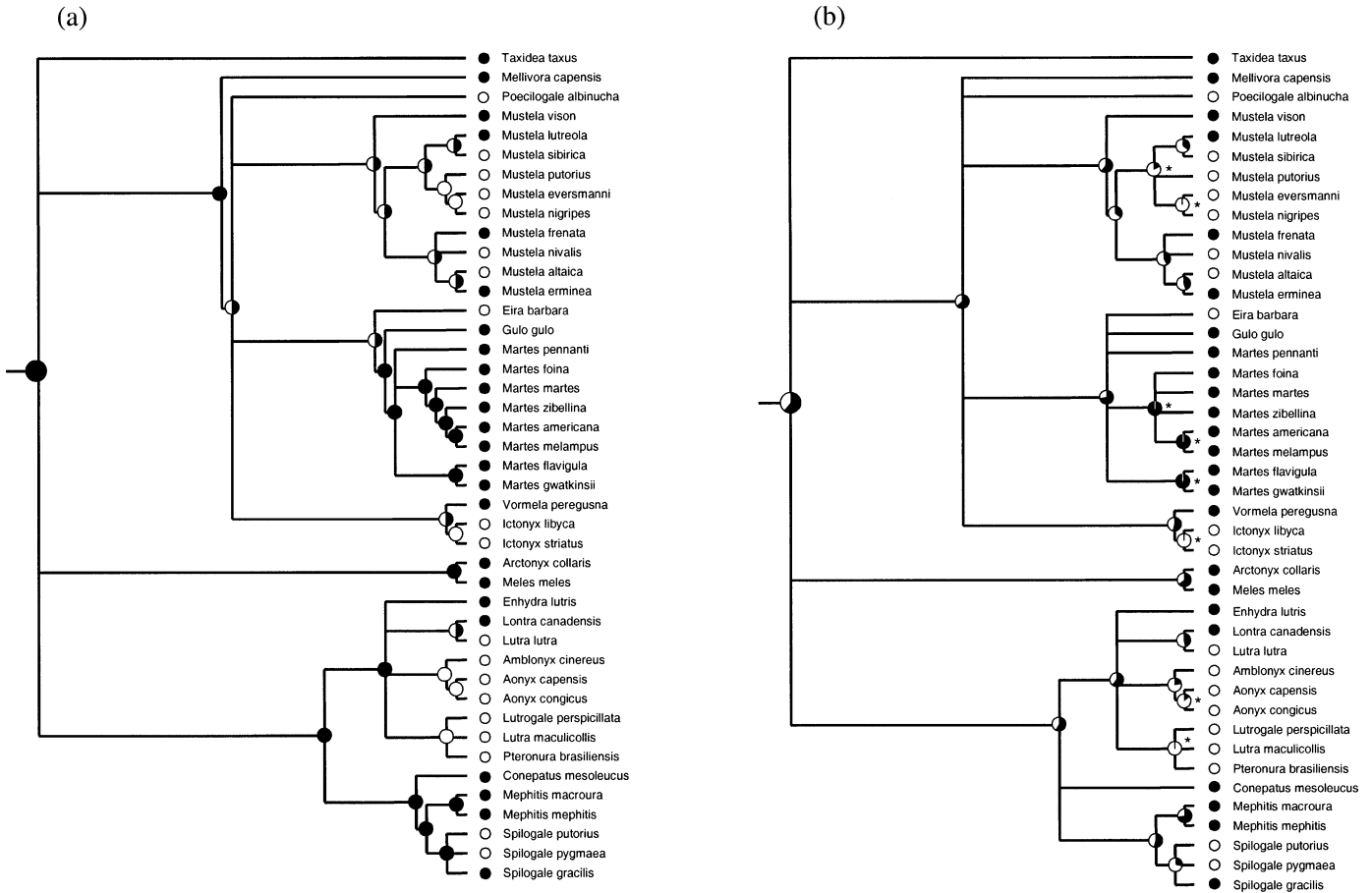


FIG. 1. Ancestral character state reconstruction for delayed implantation (DI) in the Mustelidae. The phylogeny is based on the supertree of Bininda-Emonds et al. (1999), branch lengths are not to scale. Seventeen known mustelid species with missing data on the state of DI are omitted. The state of each extant species is shown at the tree tips: ●, DI present; ○, DI absent. (a) Maximum parsimony reconstruction based on the original polytomous tree. Ancestral node symbols as for species, except ○ represents ambiguous reconstruction. All putative branches shown, including those estimated to be of length zero or negative. (b) Maximum likelihood (ML) reconstruction:  $\alpha = \beta \approx 0.05$  to 0.1,  $\kappa \approx 0.58$ . The relative support for one state over the other is represented as proportion of shading in the pie diagram. Significant support (more than two log units difference in ML, indicated with an asterisk) lies between 11 and 12 o'clock and 12 and 1 o'clock on the pie diagrams, for presence (black) and absence (white) of DI, respectively, and is marked with an asterisk. We used a randomly resolved tree for this analysis, with originally polytomous nodes separated using zero-length branches. However, all such nodes within each original polytomy had virtually identical likelihoods, so for simplicity only a single value is shown at the original polytomous node.

change more in longer branches, indicating that more recent evolution contributes more than earlier events (Pagel 1999a).

We used a composite phylogeny of the Mustelidae (Bininda-Emonds et al. 1999), a supertree derived via matrix representation with parsimony analysis from 30 source trees. Species with unknown character state information were excluded. This involved the removal of 23 species, leaving 43 with known presence or absence of delayed implantation. Among these is the western spotted skunk, *S. gracilis*, which has recently been recognized as a separate species (Verts et al. 2001) and thus does not occur on the original phylogeny (Bininda-Emonds et al. 1999). We created a polytomous *Spilogale* clade by assuming that *S. gracilis* diverged simultaneously with *S. putorius* and *S. pygmaea* (Fig. 1; Dragoo et al. 1993).

We used MacClade ver. 4.03 to find the most parsimonious set of ancestral states, first using the original polytomous tree. This method resolves polytomies in the way that minimizes the number of state changes across the tree (Maddison

and Maddison 2001). The polytomies in the mustelid tree represent uncertainty in node resolution (soft polytomies), rather than simultaneous speciation events. Because the presence of soft polytomies can produce unreliable character state reconstructions (Maddison and Maddison 2001), we resolved the tree randomly using MacClade. This involves sampling randomly from the set of all possible bifurcating resolutions of each polytomous node, with each tree being considered equally probable. We reconstructed character evolution in 100 random trees, with transformations between states either delayed or accelerated (DELTRAN and ACCTRAN, respectively, in MacClade; Maddison and Maddison 2001). These algorithms favor parallelisms and reversals, respectively, and illustrate the effect of character transition rates where there are several equally parsimonious optimizations of a character.

ML analyses were performed using the program Discrete (Pagel 1994, 1999a,b). We used a single random resolution of the tree for the ML analyses; polytomies were reduced to bifurcations by including intermediate zero-length branches



(this minimizes the effect of these branches on the resolution). We found that manipulating this minimum branch length had little effect on the rate estimates. Both one- and two-rate models were tested (i.e.,  $\alpha = \beta$  and  $\alpha \neq \beta$ ), and various values of  $\kappa$  were investigated. ML methods, such as that employed by Discrete, compare various models of evolution and estimate their likelihood given the data. Although likelihoods do not always translate directly into statistical significance levels, a difference of more than two log units is regarded as a rule of thumb by which a significant relationship may be inferred (Pagel 1994, 1999b). We used a difference of more than two log units to establish preference for one state or model over another.

#### *Life-History Variables*

The data used here are based on those of Johnson et al. (2000), to which we added geographical data from the literature (see Appendix available online at <http://dx.doi.org/10.1554/03-062.1.s1>). Measurements were taken from wild populations wherever possible, with the notable exception of longevity, which came from a mixture of wild and captive studies. Gestation is the time between fertilization and parturition (i.e., including the period of delay); we refer to the period between implantation and parturition as the postimplantation period (PIP). The term PIP + lactation period (both periods measured in days) was used as a measure of maternal investment in offspring. We scored species as having or not having DI on the basis of accounts in the journal *Mammalian Species* and the primary literature. The great majority of reports rely on identification of dormant blastocysts in laboratory or wild specimens; a small number of studies used more indirect measures, such as divergence from the allometric relationship between body size and gestation length across placental mammals (e.g., Ben-David 1998). We recognize that for a few species there has been some difficulty in accurately calculating delay length, as indicated by the fact that in a small number of cases DI is known to occur, but the length of delay has not been determined. However, the assignment of presence or absence of DI is unlikely to be affected. We found data on the state of DI for 43 of the 60 currently recognized mustelid species. All response data were transformed using the natural logarithm, to correct for nonlinearity.

#### *Analyses of Raw Data*

We conducted principal components analyses (PCA) on various combinations of variables to summarize the variance in life-history data. Cases with missing values were omitted. PCA scores were phylogenetically controlled for analysis (see below).

Separate linear regressions were performed for each life-history variable, with delay length as the response variable. Logistic regressions were conducted using presence or absence of delay as the response variable. Body size differences between species were controlled for by entering female body mass first into a multiple regression. We eliminated species for which female mass data were unavailable.

#### *Comparative Analyses*

Raw species data are not necessarily independent, because related species may share characteristics due to common ancestry as well as through convergent adaptation (Felsenstein 1985; Harvey and Pagel 1991). We conducted phylogenetically controlled analyses using a generalized least squares approach (GLS: Martins and Hansen 1996, 1997; Pagel 1997) based on Bininda-Emonds et al.'s (1999) phylogeny (see Fig. 1). Analyses were performed using the program Continuous (Pagel 1997, 1999a), which controls for nonindependence among species by reference to an internal matrix of expected covariances among species derived from the length of shared branches.

One advantage of using GLS is the ability to estimate the rate and mode of evolution. The scaling parameter  $\lambda$  can be used to assess whether traits have a phylogenetic component. Where  $\lambda = 0$ , phylogeny does not constrain the evolution of traits, and where  $\lambda = 1$  traits evolve according to Brownian motion. Intermediate values of  $\lambda$  indicate evolution according to non-Brownian processes. As described earlier for ancestral state reconstruction, the parameter  $\kappa$  estimates the effect of branch length on trait evolution (Pagel 1999a). Independent contrasts analysis (e.g., Harvey and Pagel 1991) is a special case of the GLS approach, where all scaling parameters are set to one (Pagel 1999a). In such a case, estimates of the evolutionary regression coefficient are identical. We allowed the model to select ML values of  $\lambda$ , but did not investigate the effect of the branch-length scaling parameter  $\kappa$ .

We controlled for female mass using partial correlation, based on the correlation coefficients derived for all three variables (DI duration or binary state, female mass, and the  $y$  variable under consideration). Partial correlations were calculated manually, with  $n - 3$  degrees of freedom (Sokal and Rohlf 1981). Sample sizes differed among tests according to the pattern of missing data (see Appendix). We treated PCA scores, calculated from raw variables, like any other variable when controlling for phylogeny.

## RESULTS

#### *Ancestral Character States*

The MP reconstruction using the polytomous tree suggested multiple acquisitions of direct development from an ancestor with DI. There were 10 ambiguous nodes in this reconstruction (Fig. 1). In 100 randomly resolved trees, DELTRAN reconstructed on average 11.05 ( $\pm 0.09$ ) changes to direct development and only 0.19 ( $\pm 0.04$ ) reversions to DI (found in only 19 trees). The ACCTRAN model reconstructed slightly fewer acquisitions of direct development (7.59  $\pm$  0.09) and a slightly larger number of reversions to DI (3.65  $\pm$  0.07). In all cases, the root exhibited delayed implantation, and the character changed state on average 11.24 ( $\pm 0.06$ ) times. These reconstructions were derived from a mean 108 ( $\pm 8.16$ ) most parsimonious resolutions of the bifurcating tree.

The two-rate ML model ( $\alpha \neq \beta$ ) provided only slightly better fit than the one-rate model (difference 0.47 log units), so we used the simpler one-rate model for subsequent calculations (Pagel 1999b). In fact, with comparatively small

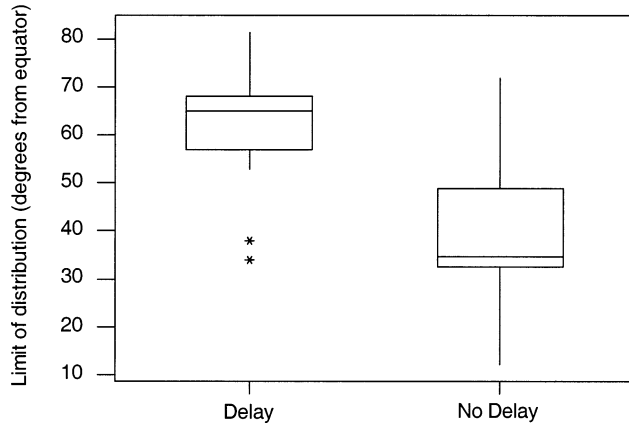


FIG. 2. Limit of geographic distribution of species with and without delayed implantation. Boxes represent the interquartile range, whiskers show range excluding outliers, horizontal line represents the median. Outliers, represented by an asterisk, are *Mephitis macroura* (upper) and *Conepatus mesoleucus*.

datasets such as ours the one-rate model is generally to be preferred (Moors and Schluter 1999). The ML estimate of  $\alpha$  and  $\beta$  was between 0.05 and 0.1; we allowed  $\kappa$  to take its ML value ( $\approx 0.6$ ), which had nonsignificantly higher likelihood than  $\kappa = 0$  (difference  $\approx 1.9$  log units). Significant evidence for one state over another was found for only nine internal nodes, all of which were close to the tree tips. The model with the root node exhibiting DI had nonsignificantly ( $\approx$ one log unit difference) higher likelihood than the alternative.

The MP and ML methods were in broad agreement about the state of most nodes. Although many nodes deep in the tree were ambiguous under both models, multiple gains of direct development (the derived condition if DI is ancestral in the family) and subsequent losses (reversion to expression of DI) were reconstructed throughout the tree.

#### Analyses of Raw Data

None of the raw life-history variables were significant predictors of nonzero delay lengths after controlling for female mass ( $P > 0.120$  in all cases).

A binary logistic regression was performed using the same variables, with delay presence/absence as the response. After controlling for female mass, PIP + lactation (representing maternal investment) was significantly negatively associated with the presence of delay (partial  $Z = -2.19$ ,  $P = 0.028$ ,  $n = 26$ ). Species without DI had a mean total PIP + lactation of  $131.5 (\pm 15.9)$  days, compared with  $99.7 (\pm 5.4)$  days for species exhibiting the trait. Maximum latitude was significantly positively associated with presence of DI after controlling for female mass (partial  $Z = 3.03$ ,  $P = 0.002$ ,  $n = 33$ ). The limit of the geographical distribution (either north or south) for species with DI was on average  $61.55 \pm 2.77$  degrees from the equator, whereas for those without DI the geographical range limit was  $39.18 \pm 4.13$  degrees (Fig. 2). Both relationships remained significant after sequential Bonferroni adjustment (Rice 1989). Although longevity was uncorrelated with delay presence/absence (partial  $Z = 1.68$ ,  $P = 0.093$ ,  $n = 30$ ), the relationship became significant after

removal of the stoat (partial  $Z = 2.14$ ,  $P = 0.033$ ,  $n = 29$ ), an extreme outlier. No other variables were significantly associated with the binary variable.

#### Phylogenetically Controlled Analyses

All models incorporating ML estimates of  $\lambda$  were significantly preferred over models where  $\lambda$  was set at zero, supporting the use of phylogenetically controlled analyses. Where  $\lambda$  is reported here,  $\lambda \approx 1$  means not significantly different from one (and the results are thus equivalent to those obtained by analysis of independent contrasts, and support a Brownian model of evolution: Pagel 1997), other reported values of  $\lambda$  are significantly different from both one and zero.

In analyses of each life-history variable against delay length,  $\lambda$  was not significantly different from one, supporting the hypothesis that the phylogeny accurately describes the variation in duration of DI among species. Longevity was negatively correlated with delay length after controlling for female mass (Table 1). However, this relationship proved to be entirely due to a single outlier (the stoat *M. erminea*); after removal, no relationship existed. No other life-history variables were significant predictors of delay length.

The model with the binary response variable produced ML estimates of  $\lambda$  at between 0.65 and 0.85; these were significantly different from both zero and one (all  $P < 0.001$ ). This indicates an intermediate degree of phylogenetic independence in the trait; DI is more independent among species than predicted from the phylogeny, but there is nevertheless an underlying phylogenetic signal (Pagel 1997). Allowing  $\lambda$  to take its ML value, only maximum distance from the equator was significantly associated with presence of DI (Table 1). However, longevity was significantly associated with DI with the stoat removed from the model (Table 1). Forcing  $\lambda = 1$  (the independent contrasts model) did not affect the outcome of the analyses.

A PCA incorporating only longevity, maximum latitude, and PIP + lactation produced two components explaining 80% of the variance in the data (Table 2). After controlling for phylogeny, the first component was significantly positively affected by longevity and PIP + lactation and negatively by latitude and the second was positively associated with longevity. A scatter plot of the first two components produced complete separation into those species with and without delayed implantation (Fig. 3).

After controlling for phylogeny and female mass, there was a significant difference between delaying and nondelaying species on both components (PC1:  $r = -0.505$ ,  $t_{19} = -2.55$ ,  $P = 0.02$ ,  $\lambda \approx 0.79$ ; PC2: partial  $r = 0.683$ ,  $t_{19} = 4.07$ ,  $P = 0.001$ ,  $\lambda \approx 0.78$ ). This implies a significant effect of longevity on the presence of DI. Excluding the very short-lived *M. erminea* from the analysis marginally increased the effect of both components (PC1: partial  $r = -0.651$ ,  $t_{18} = -3.64$ ,  $P = 0.002$ ; PC2: partial  $r = 0.692$ ,  $t_{18} = 4.07$ ,  $P = 0.001$ ).

After controlling for phylogeny and female mass, neither component was significantly associated with nonzero delay length (both  $P > 0.11$ ; both  $P > 0.84$  with stoat removed).

TABLE 1. Relationship between log-transformed life-history variables and delay length and delay presence/absence. Variation due to female mass removed, partial correlation coefficients are shown. Species with no delay excluded from the analysis of delay length. Maximum likelihood estimate of  $\lambda$  not significantly different from one for all analyses with delay length as response, all  $\lambda$  significantly different from both zero and one ( $0.65 < \lambda < 0.85$ ) for analyses with the binary variable as response. Significance of partial correlations: \* $P < 0.05$ , \*\* $P < 0.005$ .

Variable	Continuous variable (delay length)			Binary variable (delay presence/absence)		
	Partial $r$	$t$	df	Partial $r$	$t$	df
Female mass	0.120	0.500	17	0.060	0.35	35
Male mass	-0.193	-0.760	15	-0.020	-0.11	31
Mass ratio	-0.064	-0.250	15	-0.040	-0.22	30
Home range size	0.105	0.352	11	-0.224	-0.92	16
Postimplantation period (PIP)	0.042	0.168	16	-0.209	-1.19	31
Litter size	-0.110	-0.442	16	0.036	0.20	32
Lactation period	-0.251	-0.859	11	-0.127	-0.62	23
Age at sexual maturity	-0.450	-1.669	11	-0.093	-0.41	19
Longevity	-0.696	-3.497**	13	0.272	1.47	27
Longevity (stoat removed)	-0.070	-0.245	12	0.440	2.50*	26
[Maximum latitude]	0.038	0.108	15	0.550	3.43**	29
PIP + lactation	0.041	0.137	11	-0.252	-1.25	23

## DISCUSSION

### Ancestral State of Delayed Implantation

Diapause, in various forms, is an extremely widespread ecological tactic. Whether this derives from historical or contemporary selective pressure remains unknown for most groups. Resolving this question is significant, however, as trait distribution across the phylogeny informs our interpretation of its current adaptive importance under different ecological conditions (Forbis et al. 2002). Many authors have noted that the patchy distribution of DI among mammals implies multiple acquisitions (Conaway 1971; Aitken 1977; Vogel 1981; Mead 1989, 1993; Sandell 1990), although the pattern of trait evolution has not explicitly been tested for mammals. The data presented here support this interpretation, a result that was robust across a range of analyses. Nevertheless, it seems probable from our ancestral state reconstruction that the common ancestor of the mustelid family bore the trait (see Latitude below), meaning that it has changed state several times. Together these factors indicate a high degree of lability for DI in mustelids.

Many life-history traits are relatively labile in evolutionary history (e.g., coloniality in birds: Rolland et al. 1998; viviparity in iguanids: Schulte et al. 2000; parental care in birds: Leisler et al. 2002; host specialization in cuckoos and allies: Rothstein et al. 2002), and this appears to be the case for DI also. Although DI might initially be considered rather different, being a physiological trait under hormonal control, the pathways may not be particularly complex and could be

latent in nondelaying species. For instance, blastocysts of *M. putorius*, a nondelaying species, survive when delay is artificially induced (Foresman and Mead 1978). The variable delays in species such as *M. vison* and *S. gracilis* indicate that, at least in these species, there is some flexibility in the endocrine pathways, and experiments have demonstrated that flexibility can be induced in other species as well (Canivenc and Bonnin 1981).

The pattern of frequent change between DI and direct de-

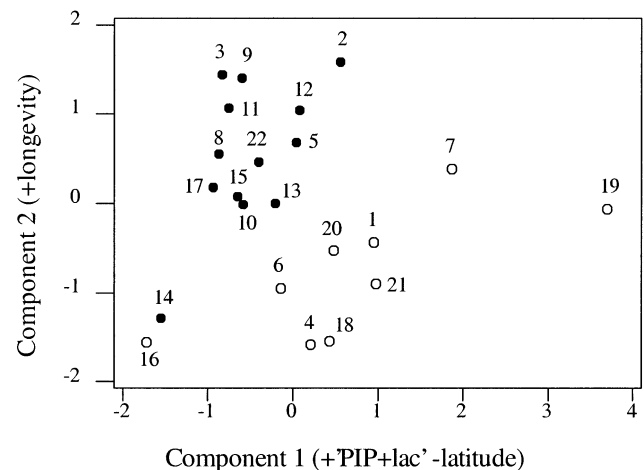


FIG. 3. Plot of principal components 1 and 2, derived from the analysis of 22 species and three variables. PC1 is strongly associated with increasing postimplantation period + lactation and decreasing distance from the equator, and PC2 represents increasing longevity (see Table 2). The data as they are presented here have not been corrected for phylogeny or female mass; however, after such correction, there is a significant difference in PC2 scores of species with and without delayed implantation (DI), but no such difference exists for PC1 scores. Closed circles, species with DI; open circles, species without DI. Species codes: 1, *Aonyx capensis*; 2, *Enhydra lutris*; 3, *Gulo gulo*; 4, *Ictonyx striatus*; 5, *Lontra canadensis*; 6, *Lutra lutra*; 7, *Lutrogale perspicillata*; 8, *Martes americana*; 9, *M. martes*; 10, *M. perspicillata*; 11, *M. zibellina*; 12, *Meles meles*; 13, *Mephitis mephitis*; 14, *Mustela erminea*; 15, *M. lutreola*; 16, *M. nivalis*; 17, *M. vison*; 18, *Poecilogale albinucha*; 19, *Pteronura brasiliensis*; 20, *Spilogale putorius*; 21, *S. pygmaea*; 22, *Taxidea taxus*.

TABLE 2. Factor loadings for the variables included in the principal components analysis. Untransformed variables were used.

	PC1	PC2
Eigenvalue	1.41	1.01
Cumulative variance	0.468	0.804
Postimplantation period + lactation	0.842	0.018
Longevity	0.304	0.927
[Maximum latitude]	-0.777	0.383



velopment among mustelids, and the implication of evolutionary lability, support a significant role for ecological factors in determining the present pattern of DI expression (e.g., Rolland et al. 1998; Leisler et al. 2002). The important evolutionary question is thus to identify its ecological benefits to modern mustelids.

#### *Life-History Determinants of Delayed Implantation*

Phylogenetically controlled analyses (also see below) produced ML values for  $\lambda$  of around 0.75. This indicates that trait evolution is more independent than predicted by the phylogeny (Pagel 1999a). However, with  $0 < \lambda < 1$ , shared evolutionary history remained important (although not according to a Brownian model of evolution) and phylogenetic correction was justified (Freckleton et al. 2002).

#### *Latitude*

The hypothesis that DI enables uncoupling of mating and parturition in seasonal environments led to the prediction of an association with latitude, because this mechanism is largely unnecessary at the equator, where seasonal variation is reduced. As predicted, DI was indeed significantly more common at greater distances from the equator. Similar correlations have been noted for other taxa (Renfree 1978; Bernard and Cummings 1997), and Ferguson et al. (1996) identified a relationship between DI and seasonality in North American carnivores, including some mustelids. DI may have evolved in response to the timing of breeding restrictions imposed by long winters, but is now adaptive only at extreme latitudes. This model implies that at least some modern mustelids may have inherited the trait from a common ancestor in which it evolved in response to prevailing environmental conditions, as suggested by our reconstruction. The earliest known mustelids (missing the carnassial notch on P4) are found in the early Miocene (Martin 1989), with the common ancestor of modern mustelids estimated to have been around 20.8 million years ago (Bininda-Emonds et al. 1999). However, the mid to upper-Miocene climate was probably subtropical or warm temperate in Eurasia (e.g., Ivanov et al. 2002), contradicting this model. Possible explanations are: DI in the mustelid ancestor evolved for reasons other than the optimal timing of reproductive events, the reconstruction is incorrect and DI evolved more recently, or DI arose even earlier than this and was retained in the mustelid common ancestor.

An extension of the seasonality hypothesis for DI is that a longer delay should be found where winters are longer, that is, further from the equator. However, we found no significant relationship between delay length and latitude, suggesting a more complex relationship between seasonality and delay length or an important role for additional factors. By comparison, delay in bats does increase slightly with distance from the equator (Racey 1982). Bernard and Cumming (1997) suggested that any such cross-species relationship might be confounded by subtle differences in reproductive physiology. The extent to which this affects the present analysis is doubtful because DI is currently thought to be controlled via similar mechanisms among all members of the Mustelidae (Mead 1993).

#### *Longevity*

With the exception of the short-lived stoat, longevity was positively associated with the presence of DI. This relationship has not previously been observed and supports our hypothesis that the time cost imposed by DI prohibits its occurrence in short-lived species. The proximate cost may well be the number of reproductive events that can occur in a lifetime: Sandell (1990) noted that DI seems to occur exclusively in species that reproduce only once per year (we did not test this explicitly because few mustelids have multiple litters per year). A time cost to DI, preventing its occurrence in short-lived species, is not necessarily expected to influence delay duration. In support of this, we found no such correlation in our data. However, the stoat forms an interesting outlier in the longevity data: it is short lived for a mustelid, yet exhibits DI. This has been noted by previous authors (King 1983, 1984; Sandell 1984), and the reason may lie in the female stoat's peculiar habit of mating before dispersal. This guarantees successful fertilization, but requires DI to allow the females to reach maturity before they give birth.

A possible cause of uncertainty may lie in the life span figures used here being derived largely from captive animals. Thus, they need to be treated with caution. In the absence of other evidence, we have assumed that longevity in captivity is likely to be correlated with life span in the wild.

#### *Maternal investment*

We hypothesized that, because of the time cost inherent in adopting DI, females might invest more per litter to minimize the risk of reproductive failure. We found no direct relationship between our measure of maternal investment (PIP + lactation) and either the presence or the duration of DI, although the measure did prove necessary to separate species with and without DI. In fact, the raw data suggested that species with DI had significantly lower PIP + lactation values than did species without the trait. This contradicts our expectation that species with DI might invest more per offspring. Interestingly Ferguson et al. (1996) found a similar trend among North American carnivores, where maternal investment (neonate size, postimplantation gestation length) was lower in species with DI. They suggest a trade-off with increased costly life-history traits (age at maturity, longevity) associated with DI, and we agree that the spreading of reproductive risk does seem a plausible argument to explain the relationship between life-history parameters of delaying and nondelaying species.

#### *Multiple Causal Factors*

Throughout this discussion, we have assumed that the causes underlying the evolution of DI are universal among mustelids. However, this may be an oversimplification. Although it appears that longevity and distance from the equator are important factors in determining which species have retained DI, the proximate benefits may differ for different species. As a brief illustration of this, consider the long and short delays displayed by mustelids. Long delays (up to 11 months) may be of benefit because they allow females to optimize both their mating and parturition timing. But such explana-

tions do not account for short-delaying species, as these delays are unlikely to be of any value in allowing mating to take place at a preferred time of year (Aitken 1977; Flowerdew 1987). The adaptive value of short delays must, therefore, lie elsewhere. The American mink (*Mustela vison*) and the striped skunk (*Mephitis mephitis*) exhibit a short and, importantly, variable delay (Venge 1973; Wade-Smith et al. 1980). One interesting effect of DI is that it allows synchronous parturition without necessitating synchronous mating (Stenson 1985). As Racey (1979) observed of sperm storage, it is easier to synchronize postcopulation fertilization (or implantation) than it is to synchronize copulations. Assuming that females can exert some degree of mate choice, an extended mating season may benefit them where individual males are infrequently and sequentially encountered, because this allows them to extend the available time for assessing males. Both the American mink and the striped skunk exhibit this pattern, with males being widely dispersed in home ranges considerably larger than those of females (Dunstone 1993; Larivière 1999). By contrast, of the two longest-delaying mustelids, the badger (*Meles meles*) and the stoat (*Mustela erminea*), badgers typically live in groups within which mating opportunities do not appear to be limited (Creel and Macdonald 1995), whereas young female stoats often mate before they disperse (Sandell 1984). Thus, seeking a common selective force underlying the evolution or maintenance of DI, and indeed other life-history traits, may be inappropriate. Nevertheless, it seems likely that longevity and seasonality play a substantial role in the evolution of DI in the Mustelidae.

### Conclusions

Despite the fact that several authors have speculated on the ancestral state of DI in various mammalian groups, ours is the first study to explicitly test the pattern of trait evolution. This study should therefore prove very useful in explaining the evolution of DI in mammals more generally. Despite considerable uncertainty in the reconstructions, all analyses conducted here point to DI being plesiomorphic in mustelids. Under any scenario, however, DI must have independently evolved several times, suggesting strong contemporary selection and contradicting suggestions that it has no current adaptive value (e.g., Mead 1989; Sandell 1990). The correlation between increasing distance from the equator and the presence of DI supports the strongest current hypothesis for the selective benefit offered by the trait, namely that it enables mating and parturition to be temporally separated.

Furthermore, for the first time we identify a potential cost to DI. Because it is not generally supported in short-lived species, we suggest that the additional time burden generated by the trait outweighs any selective advantage it may offer. That DI is potentially costly in some manner is also supported by the likelihood that it has been lost several times since the mustelids diverged. While the variables of longitude, longevity, and to a lesser extent maternal investment, adequately explain much of the variation in DI state among modern mustelids, the factors underlying the temporal variation in delay are as yet unresolved. We consider it likely that more than a single explanation may be required to describe delay

length; this variable does not correlate in our data with longevity or seasonality, and as yet there are few other general hypotheses pertaining to variation in delay length. Further work on the short-delaying species and on unraveling the factors regulating diapause duration more broadly would be of great interest.

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