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Reliability of stained placental scar counts in farmed American mink and application to free-ranging mustelids

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Despite the numerous studies carried out on the endangered European mink (*Mustela lutreola*) for conservation purposes, the reproductive biology of this species is largely unknown. In the wild reproductive parameters such as litter size are difficult to observe, particularly for cryptic species such as mustelids. We compared the reliability of nonstained and stained placental scar counts in farmed American mink (*Neovison vison*) with known litter sizes and then applied the best methodology to free-ranging related species—European mink, European polecat (*Mustela putorius*), and feral invasive American mink—for a comparative study of embryonic litter size in western Europe populations. The staining method allowed us to improve the detection of placental scars and to increase the reliability of the method by reducing the observer effect. Nevertheless, this analysis must be performed by 7 months postpartum, before the regeneration of uterine tissues. In free-ranging animals the mean embryonic litter size, estimated by stained placental scar counts and embryo counts, was significantly lower in European mink compared with polecats and American mink, and in polecats compared with American mink. Small litter sizes in European mink could be a factor limiting population growth rates in the species. Our results constitute a first step toward demographic analyses aimed at modeling the population dynamics of these species. DOI: 10.1644/09-MAMM-A-297.1.

Key words: embryonic litter size, farmed, free-ranging, *Mustela lutreola*, *Mustela putorius*, *Neovison vison*, placental scar counts, reproduction

The distribution of the European mink (*Mustela lutreola*), a small semiaquatic mustelid, has contracted dramatically over the last century (Maizeret et al. 2002; Maran and Henttonen 1995), and the species currently is listed as endangered (i.e., facing a very high risk of extinction in the wild in the near future) by the International Union for the Conservation of Nature and Natural Resources (IUCN; IUCN 2009). Remaining populations are currently distributed in 3 well-separated populations, the first in northern Spain and southwestern France, a second one in Romania, and a third one (larger than the others) in Belarus and Russia (Maran et al. 2008). The populations are still declining in these 3 areas due to several factors including anthropic pressure (habitat loss and degradation, historical hunting, accidental trapping, dog predation, and vehicle collisions), interspecific competition with the alien invasive American mink (*Neovison vison*, formerly named *Mustela vison*), and infectious diseases (Fournier and Maizeret 2003; Maran 2007). In the last 15 years numerous studies on

habitat use (Fournier et al. 2007; Zabala and Zuberogoitia 2003), spatial behavior (Ceña et al. 2003; Fournier et al. 2008; Zabala et al. 2006), health status (Fournier-Chambrillon et al. 2004a, 2004b; Philippa et al. 2008; Torres et al. 2008), and conservation genetics (Michaux et al. 2005) have been conducted on the western population, including comparative studies with related European polecats (*Mustela putorius*) and feral American mink, to understand the causes of the decline and to propose conservation measures. However, the reproductive characteristics of free-ranging European mink and related species still remain unknown, although this comparative information would be helpful for understanding their life histories and modeling their population dynamics. European polecats are found throughout most of Europe and are listed as ''least concern'' by the IUCN, although there is a recent decreasing population trend (IUCN 2009). American mink were introduced in Europe for fur production, but subsequent escapes from fur farms and successful colonization of habitats have led to the establishment of populations in large parts of Europe (Dunstone 1993). Currently, the species is considered as invasive in several European countries (Bonesi et al. 2006).

In such cryptic species observations of litters at birth are particularly rare, even for radiotracked animals (Ceña et al. 2003), and using embryos and placental scar counts (PSC) may be the most accurate and practical methods for determining litter size (Helle and Kauhala 1995). Counts of embryos and placental scars have been used to assess female fecundity for several carnivores (Elmeros and Hammershøj 2006; Hauer et al. 2002; Kristiansen et al. 2007; Mowat et al. 1996; Strand et al. 1995). A distinct implantation site is formed for each fetus in the uterus of mammals with a zonary endotheliochorial or discoid hemochorial type of placenta (Kaufmann and Burton 1994). During parturition separation of the placenta from uterine tissues generates an imprint at each implantation site, which becomes pigmented due to the phagocytosis of placental and blood remains by macrophages (Wydoski and Davis 1961). However, PSC can either overestimate the litter size due to embryo resorption, prenatal mortality, stillborn kits, or cannibalism of kits, or underestimate it due to the postpartum regeneration of the uterine tissues. Moreover, estimation of the pregnancy rate is reliable only during the period of persistence of placental scars: after this period animals may be misclassified as barren. PSC could be helpful for estimating female fecundity, but the reliability of the method has been assessed for only a few species (Bray et al. 2003; Elmeros and Hammershøj 2006; Strand et al. 1995).

To our knowledge, placental scars have never been studied in European mink, but some data are available for related species. In farmed American mink PSC accurately estimated pregnancy rate and mean litter size up to 3 months postpartum (mPP); however, these parameters were significantly underestimated for females examined 7–8 mPP, by the end of November (Elmeros and Hammershøj 2006). Similarly, Elder (1952) noted that the scars had nearly completely resorbed before the animals were pelted in December; only 8 of 100 females known to have produced young during the spring showed remaining placental scars. These results suggest that, in mink, the regeneration of the uterine tissues occurs about 7–8 mPP, several weeks before the next breeding season. In polecats Kristiansen et al. (2007) considered that the period of detectable placental scars occurred from May to January but the authors did not describe any calibration study. We believe that the reliability of PSC at the end of the persistence period (7 to 8 mPP) could be increased by staining the uteri as described by Bray et al. (2003) in European hares (*Lepus europaeus*).

The aims of the present study were to compare the reliability of nonstained and stained PSC in farmed American mink with known litter sizes 7 to 8 mPP and determine the latest time of reliable identification; apply the best methodology for PSC on free-ranging related species, i.e., European mink, feral American mink, and European polecats; and compare embryonic litter sizes of these species via counts of embryos and placental scars.

Materials and Methods

Experimental evaluation of PSC on farmed mink.—We collected 49 uteri from American mink belonging to 3 farms in southwestern France. The animals were kept under standard farming conditions in individual cages with a nest box. All females had been mated during the spring by introducing a male to the female. From the first farm we collected 13 females in 2005 and 2006, 10 females that produced offspring (including 5 primiparous individuals and 5 that had a litter in the previous year) and 3 females for which neither gestation nor young were observed by the farmer during the year of sampling. One of these 3 females was maiden; the 2 others had a litter in the previous year. Parturition occurred from 27 April to 12 May, and the females were sampled in the pelting season between 28 November and 1 December, 7 mPP. For each female the farmer determined litter size 2 days after parturition. We removed uteri from the carcasses on the day of death and froze them in water to avoid desiccation of these fragile tissues while frozen. Our experimental evaluation needed to be performed using frozen tracts, because free-ranging collected specimens usually were frozen due to constraints of field biology. This postmortem handling method for uteri has been used commonly on various species for further PSC or embryo counts (Elmeros and Hammershøj 2006; Hauer et al. 2002; Lindström 1981; Mowat et al. 1996). In 2006, from a second farm, we collected 11 females that produced young. Parturitions occurred from 1 to 10 May, and the females were sampled on 5 December, 7 mPP. Litter sizes varied from 1 to 5 kits, but we did not know the exact litter size for each female. For the third farm we examined 25 uteri from females that produced young and died naturally during 15–31 December 2006. Parturitions occurred from 28 April to 2 May; according to the farmer, litter sizes varied from 6 to 8 kits. We did not know exact dates of death for farm #3 females, but uteri were examined 7.5 to 8 mPP. The age and reproductive histories of females were not indicated by

farmers #2 and #3. In these farms farmers' practices did not allow us to collect uteri on fresh specimens; rather, uteri were removed from thawed carcasses and soaked in water and refrozen until analysis.

We slowly thawed the reproductive tracts under tap water at ambient temperature before examining them. We removed ovaries, oviducts, mesometrium, and connective tissues from each uterus, and we conducted a first macroscopic observation. We first noted the color and consistency of the uterine horns. American mink display a discontinuous zonary endotheliochorial placenta with a central hematoma located antimesometrially (Pfarrer et al. 1999) so we cut horns lengthwise with very fine dissection scissors distally from the mesometrium to avoid damage to placental scars. We examined the uterine tissues with a dissection microscope. The proposed method for counting placental scars is based on the aspect of the uterine tissues (frame structure and color). Scars were detected by the presence of a crater breaking the lengthwise folds of the tissues and by the presence of blood residues. For each uterus we carefully recorded the position and the description of visible placental scars. We stained the uteri following the method of Bray et al. (2003). We immersed uteri for 10 min in a fresh 10% solution of ammonium sulfide (H_8N_2S) and rinsed them thoroughly in tap water for several minutes, then immersed them for 10 min in a solution of equal portions of 1% chlorhydric acid and of a 20% solution of potassium hexacyanoferrate ($K_4[Fe(CN)_6]$, $3H_2O$). As a result, macrophages filled with hemosiderine had a blue-black coloration. The solutions are corrosive so it is essential to rinse the tracts again after the second bath, to keep them wet, and to analyze them quickly after staining (<2 h). After staining, we carefully re-examined the uteri with a dissection microscope. Stained scars again were described and recorded.

Each uterus was independently examined before and after staining by 2 to 3 observers. We then calculated the differences of PSC between the different observers. When counts of placental scars differed, observers reanalyzed the uterus in question to reach a consensus regarding the count.

Estimation of embryonic litter size in free-ranging mustelids.—We used carcasses of free-ranging mustelids collected in 3 study areas throughout the year by large networks organized for several previous scientific studies (Bifolchi 2007; Fournier-Chambrillon et al. 2004b, 2007). These studies were licensed by the French Ministry of Environment and the Navarre government. Members of the networks were asked to collect all dead mustelids found fortuitously in the wild, and causes of death for European mink and polecats were mostly road collisions and kills by carnivores. American mink were trapped and killed for pest control. We noted the date of discovery or death. For this study we examined females of 13 European mink, 66 American mink, and 10 polecats, collected between 1994 and 2007 in 8 departments of southwestern France; 34 American mink collected between 2004 and 2006 in 4 departments of western France; and 8 European mink collected between 2004 and 2008 in Navarre, Spain (Fig. 1).

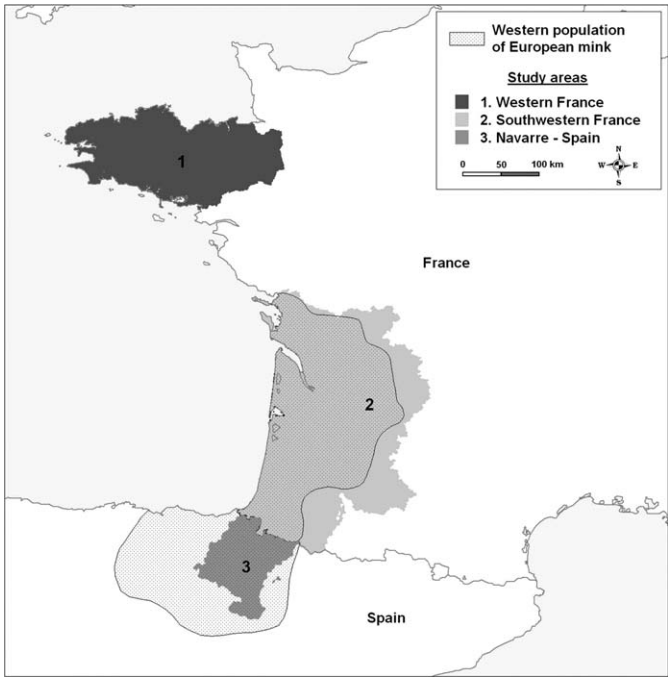

FIG. 1.—Location of the 3 study areas where free-ranging mustelids were collected for estimation of embryonic litter size using counts of embryos and placental scars. European mink (*Mustela lutreola*) were collected in southwestern France and Navarre, polecats (*Mustela putorius*) in southwestern France, and American mink (*Neovison vison*) in western and southwestern France.

For each animal we performed a detailed necropsy and examined the external reproductive organs to determine whether the females were in estrus or lactating. We removed reproductive tracts from thawed carcasses, soaked them in water, and refroze them until analyses, which were performed in series. We prepared each uterus for analysis as described above for farmed mink, and we similarly examined them before and after opening the horns.

We determined the reproductive status of females by describing the appearance of the uterine horns, which progresses according to the stage of reproduction (Helle and Kauhala 1995). During the period of sexual rest uterine horns are pale pink, and the membrane is thin with little or no lengthwise folds. When females are reproductively active uterine horns are red, thick, and rounded, and the membrane becomes thicker with more pronounced folds. After conception, uterine horns twist, until swellings start to form (early pregnancy). The swellings then become clearly apparent and can be counted accurately, with each swelling corresponding to a fetus (advanced pregnancy). At this stage the embryo count is reliable. After parturition, placental scars can be observed and counted to determine embryonic litter size. We performed PSC before and after staining as described above for farmed mink.

Data analysis.—We estimated the means of PSC, embryo counts, and litter sizes only for positive females, those that had ≥1 scar or embryo. For farmed American mink we distinguished 2 groups on the basis of the time of analysis

after parturition: females collected 7 mPP (farms #1 and #2) and females collected 7.5–8 mPP (farm #3). We compared the frequency of females with placental scars between these groups on stained and nonstained tissues, and between stained and nonstained tissues within each group, using chi-square. We compared mean differences of PSC between different observers, before and after the staining, using the Student's *t*-test. For farm #1 we tested the relationship between the PSC after staining and the breeder's counts using Pearson's correlation (r) and a linear regression analysis, with PSC as dependent variable (y) and breeder's counts as predictor variable (x). We compared the mean PSC after staining with the mean number of young observed by the farmer using the Student's *t*-test (Scherrer 1984).

To estimate the date of the earliest parturitions in free-ranging animals, we noted the dates of detection of early pregnancies (i.e., when the blastocysts are implanted) and added the duration of the postimplantation period of development. European mink and polecats have a short pregnancy period of constant duration (about 40 days), without delay of implantation of the blastocysts, whereas in American mink, the period of pregnancy is more variable (40 to 75 days) because a delayed implantation (lasting from 0 to 40 days) may be observed, depending on the date of mating (Amstislavsky and Ternovskaya 2000; Mead 1989). However, in all 3 species the postimplantation period of development is similar, lasting 27–28 days in European mink and polecats (Amstislavsky and Ternovskaya 2000) and 28–30 days in American mink (Mead 1989). The last date for reliable PSC was deduced from the date of the earliest parturitions.

In free-ranging animals we distinguished 2 methods for estimation of embryonic litter size, embryo counts on advanced pregnancies and PSC on stained uteri. Within each species we tested effects of method, study area, and method*study area on mean embryonic litter size using a two-way factorial analysis of variance (ANOVA). When differences among treatments or the interaction were not significant we pooled embryo counts and PSC and compared mean embryonic litter size between species using a single-classification ANOVA, followed by a multiple-comparison test using the Sheffé method (Scherrer 1984).

Statistical analyses were conducted using Statistica, version 8.0 (StatSoft Inc, Tulsa, Oklahoma). For all tests $P \leq 0.05$ was considered significant.

Results

Experimental evaluation of PSC on farmed mink.—In nonstained tissue visible scars were reduced to small orange-pigmented spots or somewhat diffuse brown pigmentations, sometimes associated with a crater that broke the lengthwise folds. In some cases the pigmentations were so weak that an objective count was not possible (Fig. 2a). For stained uteri, placental scars appeared as 2 orange/brown parallel bands across the horn, with a relatively pronounced central crater between the 2 bands, sometimes pigmented in brown. Various

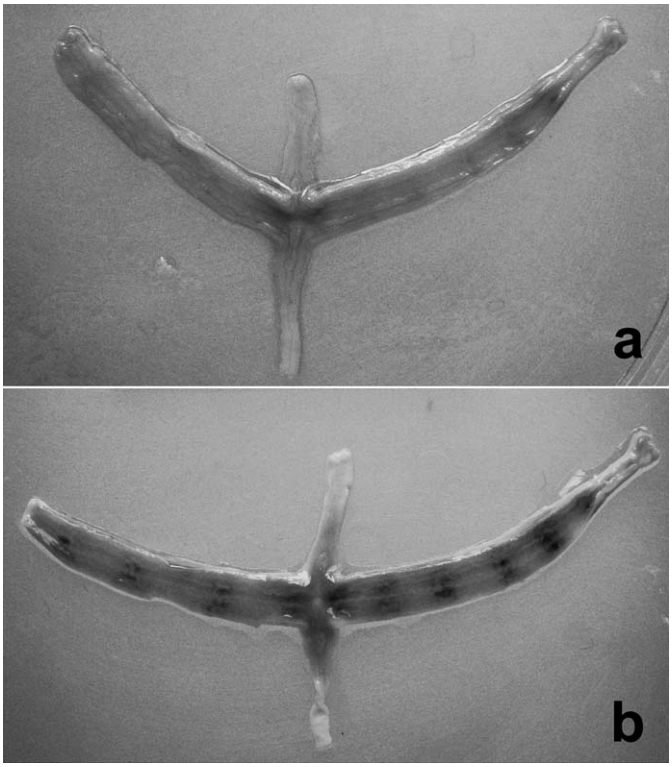

Fig. 2.—Aspect of a previously frozen uterus of a farmed American mink (*Neovison vison*) 7 months postpartum, after the horns were cut lengthwise distally from the mesometrium: (a) before staining the uterine tissues pigmentations are faint and difficult to count; (b) after staining the uterine tissues pigmented scars are clearly distinct and easy to count.

combinations of the aspect and color of the crater and the bands were possible, even on a single uterus. These scars were classified as ''Clearly distinct pigmented scars'' (Fig. 2b). Placental scars were classified as ''Scars with inconsistent stain uptake'' when pigmentations appeared after staining the tissues, but not in clearly distinguished pairs of bands as described above, so that an objective count was not possible.

Six types of results (types 1.1. to 3.3; Table 1) could be distinguished on the basis of the presence or absence of placental scars before and after staining the tissues and the type of the stained scars (clearly distinct or with inconsistent stain uptake). For 2 of the 3 females without offspring no placental scars were observed on either nonstained or stained uteri. One of these females was maiden, but the other had a litter in the previous year. For the third one, which also had a litter in the previous year, little pigmentation was observed before staining, and scars with inconsistent stain uptake appeared when staining the horns.

In females with kits analysis of tracts before and after staining the tissues failed to detect 100% of pregnancies. However, reliable PSCs were possible in significantly more females 7 mPP than 7.5–8 mPP using both nonstained tissues ($\chi^2_1 = 5.34$, $P < 0.025$) and stained tissues ($\chi^2_1 = 4.22$, $P < 0.05$). Frequency of uteri with reliable PSC was not significantly improved after staining the tissues ($\chi^2_1 = 1.87$ and $\chi^2_1 = 3.00$, $P > 0.050$, for 7 mPP and 7.5–8 mPP,

TABLE 1.—Detailed results of experimental placental scar counts on nonstained and stained uteri in farmed American mink (*Neovison vison*) of southwestern France according to the time postpartum (mPP = months postpartum).

		7 mPP (farms #1 and #2)		7.5–8 mPP (farm #3)
	Type	Females without young (n = 3)	Females that produced young (n = 21)	Females that produced young (n = 25)
On nonstained tissues: no scar After staining the tissues				
No scar	1.1.	2	3	6
Scars with inconsistent stain uptake	1.2.	0	1	1
Clearly distinct pigmented scars	1.3.	0	2	2
On nonstained tissues: little pigmentations without objective count After staining the tissues				
Scars with inconsistent stain uptake	2.2.	1	0	5
Clearly distinct pigmented scars	2.3.	0	2	4
On nonstained tissues: distinct scars easy to count After staining the tissues				
Clearly distinct pigmented scars	3.3.	0	13	7
Mean ± *SD* difference of PSC (range) between different observers before staining the tissues (type 3.3.)			2.6 ± 2.7[a] (0–7) n = 22	1.2 ± 1.3[b] (0–5) n = 14
Mean ± *SD* difference of PSC (range) between different observers after staining the tissues. (types 1.3., 2.3., and 3.3.)			0.5 ± 0.9[a] (0–3) n = 34	0.2 ± 0.4[b] (0–2) n = 27

[a,b] Mean values with the same superscript are significantly different within each time postpartum ($P \leq 0.05$).

respectively), but once stained, placental scars were counted more easily. The mean difference of PSC between different observers was significantly reduced to 0.4 ± 0.7 *SD* after staining the tissues, compared with 2.1 ± 2.3 without staining ($t_{95} = 5.34$, $P < 0.001$).

In farm #1, where exact numbers of young for each female were known, PSC after staining was positively related to the breeder's count ($y = 0.797x + 475$; $r^2 = 0.49$; $F_{1,8} = 7.80$, $P = 0.023$). The mean PSC after staining the tissues (9.7 ± 2.1) was significantly higher ($t_{18} = 4.03$, $P < 0.001$) than the mean number of young observed by the farmer (6.2 ± 1.8).

Estimation of embryonic litter size in free-ranging mustelids.—Early pregnancies were detected in 8 American mink between 2 March and 12 April, and advanced pregnancies were detected in 7 American mink between 4 April and 5 May. Because the duration of the postimplantation period of development is 28–30 days in American mink (Mead 1989), the earliest parturitions likely occur at the beginning of April in this species. Advanced pregnancies were detected in 4 European mink between 16 April and 20 May, but no pregnancy at early stages was observed in this species nor in polecats; therefore an estimation of the earliest parturitions was not possible. Because the duration of the postimplantation period of development is similar, the earliest parturitions could occur at the beginning of April for all 3 species. According to the experimental evaluation of PSC on farmed mink, we considered that PSC of stained tracts remained reliable until 7 mPP; thus, the last date for reliable PSC on free-ranging species was near the end of October. Therefore, females collected from November onward were not included in the estimation of embryonic litter size, although placental scars could be detected until December.

Placental scars were observed from May through October in free-ranging animals and were easy to count, with consistent PSC before and after staining for 61.3% of the uteri (n = 31). Without staining, scars appeared mostly as 2 dark parallel bands across the uterus with a dense pigmented crater between the bands, the latter being more diffuse in European mink and polecats than in American mink (Figs. 3 and 4). Placental scars were difficult to count reliably without staining the tissues in 38.7% of the uteri. In these cases a reliable count was always possible after staining the tissues (Fig. 5). One polecat collected on 5 March had no detectable placental scars before staining, and 5 scars with little pigmentation after

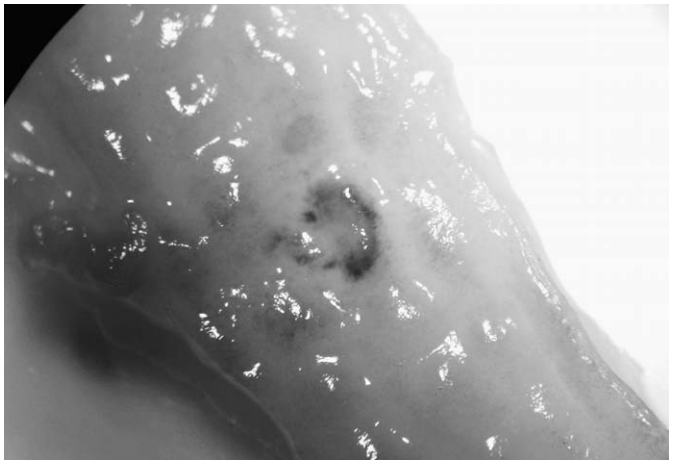

FIG. 3.—Placental scar before staining a previously frozen uterus of a free-ranging American mink (*Neovison vison*) sampled in August. The pigmented crater is clearly visible although the bands are barely visible.

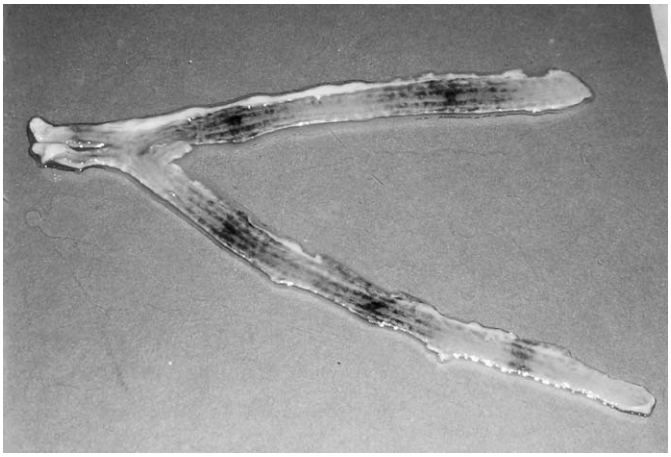

Fig. 4.—Aspect of a previously frozen uterus of a free-ranging European mink (*Mustela lutreola*) sampled in July after the horns were cut lengthwise distally from the mesometrium and before staining the uterine tissues. The scars appeared as 2 dark parallel bands across the uterus with a densely pigmented crater between the bands that are more diffuse than in American mink.

staining, suggesting mortality shortly after embryo implantation.

For each species no effects of the method (embryo counts or PSC) or of the study area were significant for mean embryonic litter size, so the data were pooled (Table 2). Significant differences in mean embryonic litter size were detected among the 3 species ($F_{2,39} = 32.97$, $P < 0.001$). Mean embryonic litter size was significantly lower in European mink compared with polecats ($P = 0.048$), in polecats compared with American mink ($P = 0.018$), and in European mink compared with American mink ($P < 0.001$).

Discussion

Experimental evaluation confirmed our hypotheses about our PSC technique. We found a complete absence of scars in a female that had whelped a litter in the previous year but did not have a litter in the year of evaluation. It appears that in these mustelids with only 1 breeding season per year (Amstislavsky and Ternovskaya 2000), scars from the previous pregnancy do not persist because uterine tissues regenerate. Similar to the study described by Elmeros and Hammershøj (2006), we found that nonstained placental scars were detectable in most, but not all, female American mink sampled 7–8 mPP. At this time postpartum we observed only pale scars or small orange-pigmented spots, corresponding to advanced tissue regeneration. Moreover, as described by Bray et al. (2003) and Elmeros and Hammershøj (2006), we also found that scars from a given uterus were not always identical, suggesting that the uterine tissues at each implantation site may regenerate at different rates. In the most regenerated states no reliable PSC was possible.

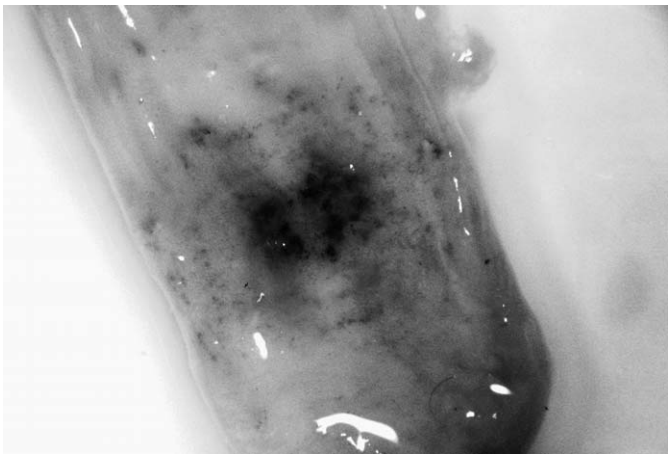

Fig. 5.—Placental scar after staining a previously frozen uterus of a free-ranging American mink (*Neovison vison*) sampled in October. The dark crater is clearly visible between the pigmented bands.

Table 2.—Estimation of embryonic litter size on free-ranging European mink (*Mustela lutreola*), polecats (*Mustela putorius*), and American mink (*Neovison vison*) from western and southwestern France and Spain, using embryo counts on pregnant females and placental scar counts on stained uteri from breeding females until the end of October.

Species	Mean ± *SD* embryonic litter size	Min–max	*n*
Mustela lutreola	$3.4 \pm 0.9^{a,b}$	2–5	12
Mustela putorius	$5.4 \pm 0.5^{a,c}$	5–6	5
Neovison vison	$7.5 \pm 1.7^{b,c}$	3–11	25

^{a,b,c} Mean values with the same superscript are significantly different ($P \leq 0.05$).

Staining of uteri increased the reliability of PSC by revealing scars that were undetectable before staining and by reducing the observer effect. The frequency of females with reliable PSC was significantly higher 7 mPP compared with 7.5–8 mPP, confirming a rapid disappearance of the placental scars due to tissue regeneration several weeks before the next breeding season. We consider that the scars with inconsistent stain uptake, mostly observed 7.5–8 mPP, might correspond to the most regenerated states. We also suggest that such scars observed in the female without young might be explained by prenatal mortality. These scars with inconsistent stain uptake were very similar to those of females with young, so in agreement with Bray et al. (2003) and Elmeros and Hammershøj (2006), we consider that pale scars should be interpreted with great caution as an indicator of postimplantation mortality.

Stained PSC overestimated litter size observed by farmer #1 2–3 days postpartum probably due to the occurrence of cannibalism of kits by the farm mink before the breeder checked the number of newborns (T. Agraffel, pers. obs.), but also due to possible prenatal mortalities, although these events could not be estimated in our study. However, uterus staining through 7 mPP remains a reliable method for estimating embryonic litter size (if not litter size at birth) and for comparative studies.

Our staining method until 7 mPP was used successfully on free-ranging mink and polecats and increased the reliability of the PSC for estimation of embryonic litter size. We recommend using this method routinely for PSC on free-

ranging animals that are collected fortuitously, especially for specimens not preserved immediately after death.

Placental scar counts used on red foxes (*Vulpes vulpes*) overestimated true litter size as indicated by counts of live embryos (Allen 1983). On raccoon dogs (*Nyctereutes procyonoides*), however, these 2 methods were in close agreement (Helle and Kauhala 1995). In our study results were similar for all mustelid species examined with regard to embryo counts and stained PSC up to 7 mPP.

To our knowledge, data on the litter size of free-ranging European mink from the western population are very scarce. Camby (1990) mentioned 3 pregnant females from southwestern France with 7, 6, and 6 embryos, but our results are inconsistent with their data because we never observed more than 5 placental scars. Our results (2 to 5 embryos) are more in agreement with the data of Ceña et al. (2003), who observed an average of 1.82 kits (range: 1–4) about 1.5–2 months old on 6 radiomonitored females in Spain. Our observations in the western European population also suggest smaller litters than those observed in the eastern European population; an average litter size of 4.3 kits (range = 1–9) has been mentioned in free-ranging European mink, and of 4.6 ± 0.1 kits (n = 274, range = 1–9) in captivity (Amstislavsky et al. 2004; Amstislavsky and Ternovskaya 2000). Small litter sizes in western European mink could be a factor limiting their population growth rates.

Multiple factors can affect litter size. Reproduction can be age-related (Allen 1983; Hauer et al. 2002) or affected by food resources (Helle and Kauhala 1995), diseases (Hansen and Lund 1988), or exposure to contaminants (Aulerich and Ringer 1977; Wren 1991). In the western population of European mink influence of age, food resources, or exposure to contaminants were not studied, but reproduction could be affected by the presence of the Aleutian disease virus (Fournier-Chambrillon et al. 2004a), which can induce spontaneous abortion and stillbirths and have an unfavorable influence on fertility. In addition, recent studies clearly pointed out the absence of genetic diversity within this western population, providing evidence of a recent bottleneck (Michaux et al. 2005). This phenomenon also could play a role in the low reproductive rates of this population. Inbreeding can reduce fecundity and increase the rate of congenital malformations, sterility, abortions, stillbirths, perinatal losses, neonatal deaths, and juvenile mortality (Jaber et al. 1998; Ryan et al. 2003). Moreover, major histocompatibility complex (MHC)-dependent mating preferences have been described and may function as a means of inbreeding avoidance. Reduced diversity in MHC might have a direct negative effect on the fitness of the fetuses (Hedrick 2003). These results are particularly alarming when considering the dramatic decline of the species during the last decades.

Our results suggest that the European mink might be jeopardized by the high fertility of the introduced American mink. The mean embryonic litter size observed in American mink in our study is similar to the results reported by Sidorovich (1993) in Belarus in an expanding population (mean ± *SD* number of embryos = 7.5 ± 0.7, n = 10). Such reproductive rates might allow rapid growth of populations in spite of local hunting pressure. Moreover, in the population of American mink inhabiting western France, admixture among differentiated gene pools could increase genetic diversity within this population and lead to an increase in adaptive potential (Bifolchi et al. 2010). The coexistence of the invasive American mink with the native European mink is particularly disturbing due to the possible occurrence of competition, possible transmission of diseases, and particularly nontarget culling (Fournier and Maizeret 2003). Matings between the 2 species, only observed in captivity, could also occur, but introgression is impossible because these crossings lead to resorption of hybrid embryos in the late stage of pregnancy (Amstislavsky and Ternovskaya 2000; Wirth 1990). However, these matings could constitute a further cause of decline for the endangered European mink due to loss of breeding opportunities (Maran and Henttonen 1995), which is particularly harmful to this species because induced ovulation permits only 1 breeding per year (Ternovsky and Ternovskaya 1994). Our results highlight the urgent need to define efficient strategies for the control and limitation of the expansion of the invasive American mink.

Embryonic litter size of European polecats was intermediate between European mink and American mink and slightly lower than the mean litter size of 6.15 young ± 0.16 *SE* (n = 203) reported previously in Russia and the average of 5.95 ± 0.62 scars (n = 18) for Danish polecats (Kristiansen et al. 2007). However, our results should be confirmed with a larger sample.

Our study constitutes a first step toward an enhanced understanding of reproductive parameters of these 3 mustelids. Further comparative studies on pregnancy rate and age-related fertility would allow a better understanding of the different population dynamics of these species. These types of information could be integrated into population viability analyses to improve conservation planning for the endangered European mink.

Résumé

Le Vison d'Europe (*Mustela lutreola*), petit Mustélidé en voie de disparition, a fait l'objet de nombreuses études scientifiques ces dernières années afin de proposer des mesures de conservation adaptées. Toutefois, sa biologie de la reproduction reste largement méconnue. L'observation de portées à la naissance pour définir le nombre de jeunes est particulièrement difficile chez des espèces aussi discrètes. Les objectifs de notre étude étaient de comparer la fiabilité du décompte des cicatrices placentaires avant et après coloration spécifique chez des Visons d'Amérique d'élevage (*Neovison vison*) au passé reproducteur connu et d'appliquer la méthode la plus fiable à des spécimens de Vison d'Europe, putois (*Mustela putorius*) et Vison d'Amérique sauvages de l'Europe de l'Ouest, afin de réaliser une étude comparative de leur portée embryonnaire. Nos résultats ont montré que la

coloration spécifique des utérus permettait d'améliorer la détection des cicatrices placentaires et augmentait la fiabilité du décompte en réduisant l'effet observateur. Le décompte ne peut cependant être réalisé que jusque 7 mois post-partum, avant que la muqueuse utérine ne se régénère totalement. Chez les spécimens sauvages, la taille moyenne de portée embryonnaire, estimée par le décompte des cicatrices placentaires colorées associé au décompte d'ampoules fœtales chez les femelles gravides, s'est révélée significativement différente entre les 3 espèces, le Vison d'Europe ayant la taille de portée la plus faible et le Vison d'Amérique, espèce invasive, la plus élevée. Cette très faible taille de portée embryonnaire pourrait constituer un facteur limitant pour la dynamique de population de l'espèce native. Nos résultats constituent une première étape pour les analyses démographiques des espèces étudiées qui sont essentielles pour la modélisation de leur dynamique de population et la proposition de mesures de conservation ou de contrôle adaptées à chaque espèce.

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