MATERNAL DENNING BEHAVIOR AND SURVIVAL OF JUVENILES IN OPOSSUMS IN SOUTHEASTERN NEW YORK

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Maternal condition, denning behavior, and juvenile survival of 12 radiocollared Virginia opossums (*Didelphis virginiana*) were studied in southeastern New York, in the northernmost region of this species' distribution. Reproductive patterns of opossums observed in this study reflected the energetic demands of severe winter weather. Female opossums entered the breeding season in poor condition, but increased body weight by 11% while nursing their offspring. Litter size was correlated with a hind-leg fat index, a measure of the nutritional status of females. Locations of daytime dens of females indicated that all weaning dens were located in burrows and that den fidelity was greater during weaning than during nonweaning periods. Weaning dens were located in more densely vegetated habitats than nonweaning dens. Survival of juveniles prior to dispersal from the area in which weaning occurred was 22.3%, with most mortality following weaning. Increased den fidelity and use of burrows in dense cover for weaning dens are likely to increase juvenile survival.

Key words: *Didelphis virginiana,* opossum, maternal condition, denning behavior, survival

The Virginia opossum (*Didelphis virginiana*) is the only extant member of the marsupial family Didelphidae in the North American temperate zone. This opossum is a relatively recent immigrant from the Neotropics, having invaded North America since the Pliocene, when a land bridge joined the two continents (Gardner, 1973). During the past century, the distribution of *D. virginiana* in eastern North America has expanded as far north as southern Ontario, New York, Vermont, New Hampshire (Gardner, 1982), and southern Maine (Godin, 1977). Although these northernmost records are somewhat beyond the −7°C January isotherm, proposed by Tyndale-Biscoe (1973) as the approximate northern limit of the opossum's distribution, additional range expansion northward seems unlikely because opossums are poorly adapted for northern winters. The pelage of opossums is sparse, even in winter (Hsu et al., 1988), and their naked tail and ears are frequently frost-bitten. Opossums do not hibernate (Tyndale-Biscoe, 1973), but they seldom leave their dens at temperatures below −7°C (Wiseman and Hendrickson, 1950). To survive low temperatures, opossums apparently remain inactive and rely on metabolism of limited body stores.

The reproductive patterns of Virginia opossums living in northern regions of their distribution are shaped by the constraints of a short growing season and an inherently long interval (3.5 months) from conception to weaning of young. Females usually raise only one litter, whereas two smaller litters per year are common in southern latitudes

(Edmunds et al., 1978; Hartman, 1928; Lay, 1942). Litter size also appears to be related to nutritional condition in opossums (Cerqueira, 1984; Eisenberg, 1988; Motta et al., 1983); however, the relationship might not be evident if condition is assessed from body weight alone (Austad and Sunquist, 1986; Burns and Burns, 1957). Because the breeding season in New York begins in late winter and early spring (i.e., at the close of the most energetically demanding time of the year), the relationship between maternal nutritional status and litter size, which varies from three to 13, is of particular interest.

Young Virginia opossums remain in the pouch for the first 70 days of life, firmly attached to teats for the first 50 days (Gardner, 1982). During this time protection of the young is nearly complete, albeit passive, by virtue of the pouch. However, beginning at 70 days of age, the young are left in the den while the mother forages (Gardner, 1982); thereafter, maternal care and protection of the young become more complex and less certain. After weaning (93–103 days), young must forage outside the den where they are increasingly vulnerable to predation. Indeed, more than 60% might die during the first 30 days after weaning (Wright, 1989). Also, most juveniles do not disperse from the weaning area until the winter mating season, with only gradual expansion of the home range occurring in the first 3 months following weaning (Gillette, 1980; Wright, 1989). Therefore, quality of the habitat (e.g., food availability and protective vegetative cover) surrounding the weaning den should be an important determinant of juvenile survival.

Didelphis virginiana is considered to be a habitat generalist (Allen et al., 1985; Blumenthal and Kirkland, 1976; Fitch and Sandidge, 1953) and also is known to change dens frequently (Allen et al. 1985; Fitch and Shirer, 1970). In fact, dispersal of females with small pouch young (<60 days of age) also appears to be a common occurrence (Gillette, 1980). Nevertheless, opossums nursing young might, near the time of weaning, select dens in habitat favorable for survival of foraging young. Support for this notion is seen in seasonal shifts in habitat preferences of the Virginia opossum (Seidensticker et al., 1987) and prolonged occupancy of dens by females, but not males (Sunquist et al., 1987). However, little is known of habitat features that might influence selection of den sites by females during weaning or behavioral changes that might occur in females at weaning.

Because females seldom live through more than one reproductive season or produce more than one litter per year in northern regions, survival of juveniles is a critical determinant of lifetime reproductive success in opossums. Little is known about survival of juvenile *D. virginiana* in the north, but recent studies of *D. marsupialis* in Venezuela (Austad and Sunquist, 1986; Sunquist and Eisenberg, in press) and *D. virginiana* in central Florida (Wright, 1989) have advanced our understanding of survival of juveniles and encouraged comparable studies of populations of opossums in northern regions.

This study had three major objectives. The first was to determine the relationship between litter size and maternal condition in female opossums (*D. virginia*). We hypothesized that litter size would be positively correlated with maternal fat reserves. The second objective was to examine denning behavior of females and habitat characteristics of den sites for evidence of maternal care. We predicted that denning behavior and habitat at den sites would change during weaning. The third objective was to measure neonatal and juvenile survival in *D. virginiana* living near the northern limit of this species in southeastern New York.

Materials and Methods

This study was conducted on the Cary Arboretum and adjacent properties in Dutchess Co., New York, ca. 2 km W Millbrook (41°48′N; Fig. 1). This area is located 65 km south of the −7°C January isotherm (United States Department of

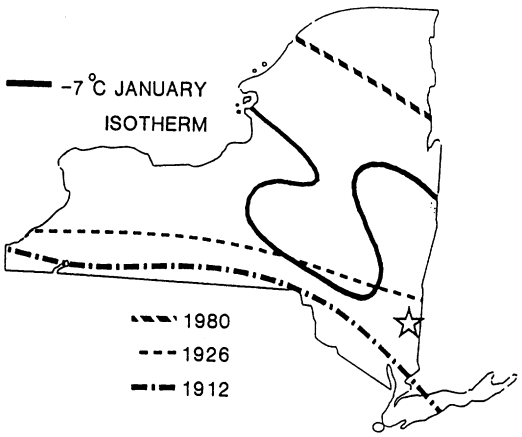

FIG. 1.—The −7°C January isotherm and changes in the northern distributional limits of the opossum in New York. Star indicates location of study area. Distributional limits adapted from Gardner (1982).

Commerce, 1987; Fig. 1); the average January temperature is −5°C. *D. virginiana* was first recorded in the area in 1928 (Coleman, 1929); it is now abundant except in years following a severe winter.

Capture and physical measurements of opossums.—We captured Virginia opossums in wooden (Mosby, 1955) and wire (Tomahawk Live Trap Co., Tomahawk, WI) live traps from 25 March to 24 June 1987 and 22 March to 21 May 1988. Females were sedated with Ketamine HCL (35 mg/kg; Ketaset, Aveco Co., Inc., Fort Dodge, IA), marked with permanent ear tags, and fitted with 36-g radiotransmitter collars (Wildlife Materials Inc., Carbondale, IL). We measured chest girth just posterior to the shoulders and length of body from snout to rump along the dorsal side of the body with a meter tape. Skin and subcutaneous fat on the posterior portion of each animal's thighs were pinched and stretched away from the underlying muscles. The thickness of this fold of skin and fat was measured at two points with a caliper; these four measurements were averaged to provide a hind-leg fat index for each female. Weights of females with pouch young were calculated by subtracting the estimated weight of the litter, based on body measurements of young (Petrides, 1949), from the combined weight of the females and their litters. Pouch young were aged to within 3 days using standard developmental criteria (Reynolds, 1952), examined for gender, and individually toe-clipped (Sanderson, 1961). We aged adult females by tooth wear and eruption (Gardner, 1982) and pouch condition (Petrides, 1949). Virginia opossums were retained in a heated building until they recovered from the anesthesia, and then released at the capture site in a soft release box (a wooden box with a free-swinging door made of a flap of inner tube), which provided the opossum with a temporary sanctuary.

Using a portable, TRX-36 receiver (Wildlife Materials Inc.) and a hand-held, 2-element, yagi antenna (Telonics Inc., Mesa, AZ), we located females in their dens during daylight hours from 21 April to 15 August 1987 and 11 April to 9 September 1988. Females were recaptured prior to and after weaning young to assess survival of pouch young, juvenile independence, and maternal physical condition. We recaptured these individuals with an extended trap that employed a burlap bag as a vestibule between the burrow entrance and the trap.

Characteristics of den sites.—The Cary Arboretum previously had been mapped into 14 plant associations, which were grouped a priori to form seven broad, habitat associations: old field, shrub-brush, hardwood forest, oak-hickory forest, deciduous forest, coniferous forest, and forest. Macrohabitat at each den site was assigned to one of the 14 plant associations and to the corresponding broad habitat association.

Microhabitat characteristics were measured within a 0.04-ha circular plot with the entrance of the den as its center using techniques modified from James and Shugart (1970). These measurements, made between 15 August and 8 September 1988, reflected conditions present at weaning because leaf-out on the study area occurred in early May (C. D. Canham, pers. comm.), and weaning of all opossums in this study occurred between 3 June and 23 August. Microhabitat characteristics measured in this study were selected on the basis of habitat relationships stated in previous studies (Blumenthal and Kirkland, 1976; Lay, 1942; Seidensticker et al., 1987) and previous observations of habitat use by opossums on the Cary Arboretum. Density of short (0.0–0.5 m) and tall (0.5–1.5 m) horizontal cover was measured on photographs taken of a density board (0.5 by 1.5 m—Noon, 1981) placed 5.6 m from the den in each of the cardinal directions. Density of horizontal cover was determined by the percentage of the board obscured by vegetation. We estimated density of shrubs

(dbh ≤7.6 cm) and species composition at breast height along a north-south belt transect (2.0 by 22.6 m) centered on the den. Percent ground and canopy cover were calculated from the presence or absence of vegetation observed through an ocular tube (James and Shugart, 1970) at 10 locations along a 22.6-m east-west transect centered on the den. Vegetation ≥2 m in height was considered canopy cover; woody and herbaceous vegetation ≤1 m in height was recorded as ground cover. Distances to the nearest edge, opening, water source, and stone wall were determined within a 200-m radius of the den, an area expected to include the home range of juveniles (Gillette, 1980). An opening was defined as an area ≥50 m^2 and free of woody vegetation ≥1.4 m in height.

Survival of juveniles.—Survival of juvenile *D. virginiana* was calculated for 3 time periods based on the behavior of the young (Gardner, 1982); pouch young (7–69 days old), weaning (70–102 days old), and independence (103–127 days old). The weaning period commences when the young remain in a den while the mother forages, and ends when the young are weaned (Gardner, 1982), although the mother might continue to den with her offspring until they are ca. 113 days old. The independence period begins after the young are weaned and continues until they disperse from the weaning area.

We determined survival during the pouch-young period by the number of young in the pouch of each recaptured female. To determine survival of juveniles, we recaptured juveniles at weaning and later when independent of the female with wire live traps (Tomahawk Live Trap Co.) set for a total of 749 trapnights. Recapture efficiency was enhanced by determining locations of maternal dens through radiotelemetry. During the weaning period of each litter, 12 traps were placed in three concentric circles spaced 5 m apart surrounding the entrance of the den. We employed a similar pattern during each independence period, except traps were placed in concentric circles spaced ca. 50 m apart. Several precautions were taken to reduce trap-related mortality. Traps, which were covered with asphalt shingles for protection from weather extremes, were not set on consecutive nights following a recapture, and released individuals were observed until they entered a den, at which time 15 g of dry dog food were placed in the opening of the den.

We used a computerized version (Brownie et al., 1986) of the Jolly-Seber capture-recapture model (Jolly, 1965; Seber, 1965) to estimate survival of juveniles for the weaning and independence periods. To estimate the survival for period n, the Jolly-Seber model requires $n + 1$ sampling periods. Therefore, to obtain survival estimates for the independence period, we added an additional period based on the assumption that individuals captured in the independence period were still alive in an additional period, 1 day later. This technique calculates the same survival rate as the minimum-number-known-alive (MNA) direct-enumeration technique (Krebs, 1966).

Statistical analyses.—We analyzed parameters of females and litter characteristics with Wilcoxon signed-rank tests (Hollander and Wolfe, 1973), linear regression, and stepwise multiple regression. Differences between survival estimates were tested using the Z-test statistic (Pollock et al., 1989). Differences between denning periods were analyzed using Wilcoxon signed-rank tests. To estimate the number of different dens used and the number of den changes in each period, we calculated a weighted average use of dens for each opossum based on the length of the telemetry period and the number of dens located. All tests were two-tailed and $\alpha = 0.05$ unless otherwise noted. Analyses were performed with SAS (SAS Institute, Inc., 1985) and Minitab (Minitab, Inc., 1985).

Results

Breeding season and litter size.—Twelve female Virginia opossums nursing young were captured; all were 9–15 months of age and in their first breeding season. The mean parturition dates for the first ($n = 10$) and second ($n = 3$) litters of the year were 25 March and 1 June, respectively. The earliest and latest dates of parturition were 29 February and 3 June, with those individuals producing two litters per year having the earliest parturition dates. One female gave birth to litters of nine and 14 on 29 February and 1 June, respectively. Thus, the female's first litter was ≤93 days old when it was weaned. After giving birth to her second litter, this female remained in the den with the first litter for 19 days before dispersing.

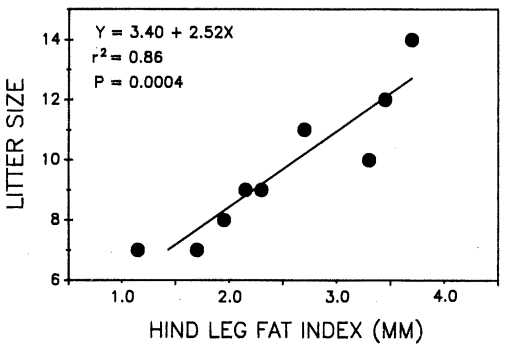

FIG. 2.—Linear relationship between litter size and hind-leg fat index of nine female opossums nursing young in southeastern New York, 1987–1988.

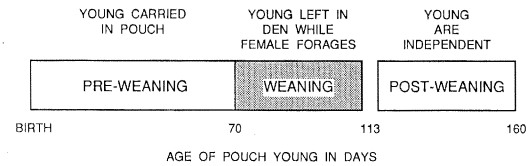

FIG. 3.—Denning periods of female opossums based on maternal-offspring associations and age of pouch young. In this study, the post-weaning period began after the female had abandoned her young (young are ca. 113 days old) and concluded when the offspring were 160 days old, the latest location of the den of a female.

Estimated dates of parturition (31 May–3 June) for two other females suggest that they were raising their second litters when initially captured and that 25% of the females produce two litters per year. The average litter size among both first and second litters was 9.4 ± 0.6.

Female *D. virginiana* gained an average of 215 ± 97 g during the lactation period; this increase (11%) was significant ($T = 41$, $d.f. = 9$, $P = 0.033$). Litter size was correlated with hind-leg fat index ($r^2 = 0.86$, $d.f. = 8$, $P = 0.0004$; Fig. 2) and chest girth ($r^2 = 0.42$, $d.f. = 9$, $P = 0.042$). A multiple regression ($r^2 = 0.90$, $d.f. = 8$, $P = 0.001$) incorporating both hind-leg fat index ($F = 27.6$, $P = 0.002$) and chest girth ($F = 2.8$, $P = 0.147$); the regression equation $Y = -1.798 + 2.156$ (hind-leg fat index) + 0.244 (chest girth) predicted litter size to within 0.6 pouch young. No correlation was detected between chest girth and hind-leg fat index ($r^2 = 0.28$, $d.f. = 8$, $P = 0.145$). Sex ratios among litters were not correlated with chest girth ($r^2 = 0.35$, $d.f. = 8$, $P = 0.093$) or hind-leg fat index ($r^2 = 0.30$, $d.f. = 7$, $P = 0.16$).

Types and uses of dens.—We obtained 271 locations of daytime dens from seven radiocollared, female Virginia opossums ($\bar{X} = 38.7$, $SE = 6.9$ locations/individual). Den-use and den-site data were analyzed by denning period based on the behavior of the female relative to the development of her pouch young (Gardner, 1982; Fig. 3). The number of locations of dens obtained during the pre-weaning, weaning, and post-weaning periods were 48, 154, and 69, respectively.

Females were located in 76 different dens, and only five (7%) dens were used by the same individual during >1 of the 3 denning periods. No dens were used by more than one female. Dens that received multiple use by an individual were recorded separately for each period used. Dens used in pre-weaning ($n = 32$) and post-weaning ($n = 23$) periods did not differ ($T \leq 24$, $d.f. = 7$, $P > 0.05$) among habitat characteristics or use; therefore, these data were combined to form a nonweaning-den category ($n = 54$) for comparison with the weaning dens ($n = 26$). A variety of types of dens was used, but 91% (73) were in burrows (primarily those of woodchucks, *Marmota monax*). Other types of dens included scrap piles, hollow logs, tree trunks, and elevated cavities in trees. All weaning dens were located in burrows, whereas 87% of nonweaning dens were located in burrows. Females used fewer different dens during the weaning period ($T = 28.0$, $d.f. = 7$, $P = 0.022$) and made fewer den changes ($T = 28.0$, $d.f. = 7$, $P = 0.046$) than during the nonweaning periods (Fig. 4). The median sojourn (length of stay) in a weaning den (5.9 days) was longer ($T = 28.0$, $d.f. = 7$, $P = 0.022$) than the sojourn in a nonweaning den (1.3 days; Fig. 4).

Characteristics of den sites.—Most dens

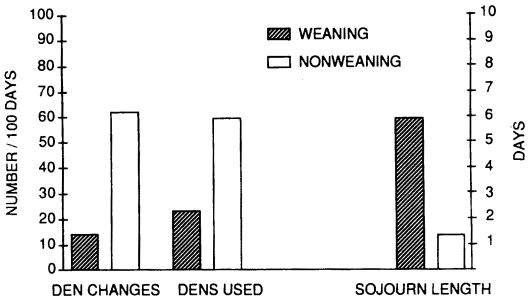

FIG. 4.—Median number of den changes, different dens used (no./100 days), and sojourn length (days) during weaning and nonweaning periods of seven female opossums in southeastern New York, 1987–1988.

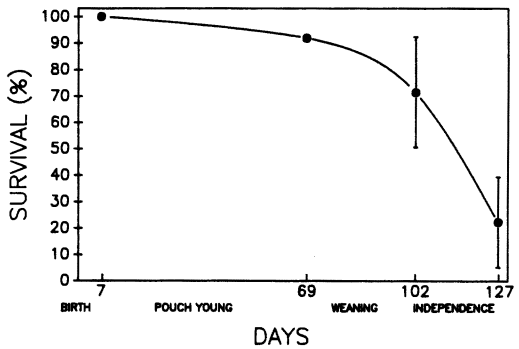

FIG. 5.—Survival curve of 85 juvenile opossums from nine litters in southeastern New York, 1987–1988. The curve is for the period between 7 and 127 days of age, which includes the pouch young, weaning, and early independence periods. Vertical bars represent standard errors.

(49%) were located in shrub-brush associations, with a greater percentage of the weaning (70%) than nonweaning (44%) dens located in these habitat types ($T = 24$, $d.f. = 7$, $P = 0.108$). More nonweaning dens (50%) were located in deciduous forests than weaning dens (21%; $T = 24$, $d.f. = 7$, $P = 0.108$). The percentage of weaning dens (2%) in sugar maple was significantly less than the percentage of nonweaning dens (24%) found in this association ($T = 27$, $d.f. = 7$, $P = 0.035$; Table 1). Few dens (7%) were located in coniferous forests, and only one nonweaning den (1%) was located in the old-field habitat association. Three of 23 microhabitat variables showed significant differences ($P < 0.05$) between weaning and nonweaning dens (Table 1); density of tall, horizontal cover ($T = 26$, $d.f. = 7$, $P = 0.046$), distance to nearest stone wall ($T = 28$, $d.f. = 7$, $P = 0.022$), and canopy cover ($T = 27$, $d.f. = 7$, $P = 0.035$). Differences in three other variables were nearly significant ($P < 0.10$): vegetative ground cover, number of witch-hazel (*Hamamelis virginiana*) stems, and distance to nearest opening. Three of these six variables are measures of vegetative cover and indicate that weaning dens were located in denser vegetative cover.

TABLE 1.—*Medians of habitat variables that differed*[a] *between weaning and nonweaning dens used by seven female opossums in southeastern New York, 1987–1988.*

Variable	Weaning ($n = 26$)	Nonweaning ($n = 54$)
Tall horizontal cover[b] (%)	59.2	41.7
Canopy cover (%)	78.1	79.7
Distance to stone wall (m)	74.4	35.7
Dens in sugar maple[c] (%)	2.4	23.7

[a] All at $P < 0.05$.

[b] Tall, horizontal cover is 0.5–1.5 m in height and is a measure of cover density.

[c] Percentage of dens in the sugar maple (*Acer saccharum*) plant association.

Survival of juveniles.—The nine litters monitored in this study contained 85 young (43 males, 42 females), seven of which died during the pouch-young period (7–69 days old), resulting in a survival rate of 91.8% (Fig. 5). Thirty-five of the remaining 78 young were recaptured as juveniles. Jolly-Seber estimates of survival during the weaning (70–102 days old) and independence (103–127 days old) periods were 77.8 ($SE = 22.8$) and 31.3% ($SE = 11.5$), respectively. The overall survival rate of juveniles (7–127 days old) was 22.3% ($SE = 17.1$; Fig. 5). Survival of juveniles did not vary significantly relative to sex, litter size, parturition date, maternal condition, year, or den fidelity. Mortality of five (6%) juveniles was trap-related.

Discussion

Litter size and nutritional status.—The average litter size (9.4 ± 0.6) observed in the present study was similar to those (8.7–9.0) in previous reports of opossums in New York (Hamilton, 1958; VanDruff, 1971), but noticeably higher than those seen in Florida (6.3—Burns and Burns, 1957) and Louisiana (6.8—Edmunds et al., 1978) where nearly all females have two litters per year. The shorter growing season in northern areas apparently favors one large litter over two smaller ones in opossums (Gardner, 1982). Virginia opossums do not breed in their 1st year (8–12 months) of life (Reynolds, 1952), and few live beyond 2 years in the wild (Fitch and Sandidge, 1953; Petrides, 1949; Sanderson, 1961; Sunquist et al., 1987). Also, reproductive performance declines sharply after the first reproductive season (year following female's birth) in both captive (Jurgelski and Porter, 1974; Reynolds, 1952) and wild populations (Sunquist and Eisenberg, in press).

In the present study, litter size was closely correlated with fat reserves ($r^2 = 0.86$) of the mother (independent of body size; Fig. 2), which supports a hypothesis set forth in the present study and confirms Eisenberg's (1988) suggestion of a positive relationship between nutritional status and litter size in *D. virginiana.* Nutritional status probably influences litter size by limiting the number of functional teats and associated mammary glands, rather than the ovulation rate or the number of young born. Virginia opossums have a high ovulation rate (ca. 60 ova/cycle—Fleming and Harder, 1983), and the number of young in utero (12–24—Hartman, 1916; Neu et al., 1977) normally exceeds the number of teats (13). Average sizes of litters usually are considerably <13 because the most anterior teats are not always fully functional (Reynolds, 1952). Young on these tend to be smaller than those on posterior teats (Cutts et al., 1978) and may suffer higher mortality, especially early in lactation.

Opossums rely heavily upon fat reserves to survive northern winters, particularly when severe weather prevents foraging (Brocke, 1970; Tyndale-Biscoe, 1973). Weight loss in winter might be independent of food availability. Captive *D. virginiana* maintained on food given ad lib. in an outdoor enclosure lost 27% of their body weight between autumn and spring (Hsu et al., 1988). Brocke (1970) estimated that about one-third of the opossum's winter energy requirements were met through catabolism of body tissues and that only 30–40% of this catabolism was derived from fat. Females captured on our study area in early spring showed signs of extreme weight loss and muscle atrophy, especially in the shoulders, back, and hind quarters. Thus, many opossums probably enter the breeding season in poor condition, and the extent to which they can find food and gain weight during gestation and early lactation might well determine their reproductive success. The 11% increase in body weight observed in females during lactation supports this notion, as does the fact that litter size was highly correlated ($r^2 = 0.90$) in a multiple regression with hindleg fat index and chest girth (an index of fat reserves and muscle).

The positive correlation between the hindleg fat index and litter size observed in the present study primarily is based on data collected early in lactation (days 7–32), which suggests that litter size appears to be determined by nutritional status (and consequently the number of functional teats) of the female at the time of birth. This conclusion is supported by the results of an experiment wherein free-ranging females were provisioned with 125 g of cat food and fish at 2-day intervals throughout the period of lactation. Provisioned females gained weight, but did not produce larger litters than control females (Sunquist and Eisenberg, in press). However, litters of provisioned females were male-biased, confirming the results of an earlier study of *D. marsupialis* in Venezuela (Austad and Sunquist, 1986). Although females in our study gained weight throughout the period of lactation, the proportion of males in litters was

not correlated with the hind-leg fat index of females in early lactation. While these results do not clarify underlying mechanisms, they suggest that litter size in *D. virginiana* is controlled by factors independent of those that influence sex ratios of litters.

Den-use behavior.—Burrows frequently were used as den sites; apparently, this is common for Virginia opossums throughout much of their range in North America (Allen et al., 1985; Hamilton, 1958; Wiseman and Hendrickson, 1950). Most of the burrows appeared to be abandoned woodchuck burrows, which were abundant and widely distributed on the study area. All weaning dens were located in burrows, suggesting that burrows might hold some advantage for juveniles over other types of dens (e.g., a constant, favorable temperature regime or protection from predators).

An increase in den fidelity during the weaning period was demonstrated by significantly fewer den changes and dens used and significantly longer sojourns. Fitch and Shirer (1970) found that den sojourns for *D. virginiana* in Kansas were longest in winter, 2.3 days; this was less than the median sojourn during the weaning period (5.9 days) in the present study. Sunquist et al. (1987) found that female *D. marsupialis* had significantly longer sojourns (5.1 days) than males (1.5 days) and attributed a portion of this fidelity to females returning to their dens to feed young. This greater den fidelity during the weaning period probably is not simply a matter of the female's immobility due to the increasing weight of her litter. One female moved her 94-day-old litter of nine (weighing >1 kg) 500 m to a new den, which is farther than the average distance Virginia opossums moved between consecutive dens in other studies (192 m—Allen et al., 1985; 299 m—Fitch and Shirer, 1970). Longer sojourns allow juveniles to become familiar with the area surrounding the weaning den prior to becoming independent and being forced to forage for themselves. Familiarity with the weaning area and the capacity for maintenance of social contact by chemical communication (Holmes, 1992) might be particularly beneficial to juvenile females, which have been shown to be philopatric (Wright, 1989). This increased den fidelity during weaning and exclusive use of burrows as weaning dens are maternal behaviors that should enhance survival of juveniles.

Characteristics of den sites.—The occurrence of dens of *D. virginiana* in some macrohabitats was not proportional to their availability on the study area. For example, old-field and shrub-brush macrohabitats covered 33 and 11% of the study area, respectively, but 1 and 49% of the dens used by Virginia opossums were located in those respective macrohabitats. These differences suggest either that some macrohabitats had a greater abundance of dens (i.e., burrows) or that female opossums selected dens in dense macrohabitats.

The use of dens in dense habitats was more pronounced during the weaning period. Fewer weaning dens were found in the sugar maple forest type, a plant association that tended to have a dense subcanopy, but lacked a shrub layer and had the lowest values of percent cover of any of the 14 plant associations. Although burrows probably were not equally abundant in all plant associations and microhabitats, the distribution of burrows did not change during the study period. Therefore, the habitat differences observed between weaning and nonweaning dens indicated that denning behavior changed when females were weaning their young. These behavioral changes, along with increased den fidelity and exclusive use of burrows during the weaning period, support the second hypothesis set forth in the present study.

Besides human causes of mortality (i.e., hunting, trapping, and collisions with vehicles) predation by owls and canids is considered the largest source of mortality among *D. virginiana* (Gardner, 1982; Hunsaker, 1977). Wright (1989) determined that, of the radiocollared juveniles that did not survive to the following breeding season, most were killed by owls while several others were killed by canids. Weaning dens located in

denser habitats should make the young less visible and accessible to these predators and thereby provide additional protection to juveniles. Most of the weaning dens in the present study were located in stands of shrub-brush species, such as Tatarian honeysuckle (*Lonicera tatarica*), which forms a dense canopy (tall horizontal cover, Table 1) ca. 1 m high that shades out much of the lower vegetation. Such areas should have provided juveniles protection from avian predators (owls and hawks, Accipitridae) and large canids (coyotes, *Canis latrans,* and dogs), and, at the same time, allowed the female and juveniles to move easily through the lower strata. Occurrence of dens used by opossums in Texas were closely correlated with wax myrtles (*Myrica cerifera*) and blackberries (*Rubus allegheniensis*—Lay, 1942), species that provide cover similar to that at weaning dens in the present study.

Didelphis virginiana frequently uses stone walls as travel routes (Stains and Baker, 1958); therefore, use of burrows close to these structures might be expected. Because the distance from dens to the nearest stone wall increased during the weaning period, other habitat features (e.g., dense cover) might be more important at this time. Similar seasonal shifts of habitat preferences were seen in live-trap data in northern Virginia (Seidensticker et al., 1987), where opossums were associated with shrubby thickets during summer (April–July), but shifted to forest edges during autumn (August–November).

Sites of weaning dens had more vegetative ground cover and fewer witch-hazel stems, and were found closer to open areas than at sites of nonweaning dens. These characteristics of weaning den sites also suggest that female *D. virginiana* use dense habitats during the weaning period. Witch-hazel is an understory shrub that grows under a closed, hardwood-forest canopy (Little, 1980) and does not persist in brushy or open areas. Although Virginia opossums tend to avoid open areas such as agricultural fields and grassland habitats (Llewellyn and Dale, 1964; Verts, 1963), they have shown a marked preference for field edges (Blumenthal and Kirkland, 1976). The shrub-brush habitat used for weaning dens frequently forms edges along open areas, and this might explain why weaning dens were closer to open areas.

Estimates for survival of juveniles.—Survival of pouch young (7–69 days of age) was high (92%) and similar to the 88–98% recorded in previous studies (Austad and Sunquist, 1986; Sanderson, 1961). However, survival was low among juveniles, especially during the first few weeks following weaning (31%). Wright (1989) observed a similar low survival rate (25–40%) within the first 4 weeks after weaning in studies of radiocollared juveniles in Florida. This short independence period is the most vulnerable period for young *D. virginiana* after parturition (Lay, 1942), because survival prior to weaning was >70% and survival following independence quickly levels off to much higher levels throughout adulthood (Wright, 1989). Survival of pouch young to their first breeding season has been estimated at 10–11% in northern Florida (Wright, 1989). However, if the additional demands of severe winter weather, as found in New York, are considered, survival of Virginia opossums to their first breeding season might be <10%.

Survival estimates derived from mark-recapture models frequently are plagued by untenable assumptions; however, in the present study, bias in the estimates for survival of juveniles was reduced because the Jolly-Seber mark-recapture model requires few assumptions and the population was closed and permanently marked as pouch young. We believe that the precautions used in the present study reduced trap-related mortality (6%) among juveniles and that the assumption of equal probability of capture (at least between sexes) was not violated. The recapture rate, important for precision in mark-recapture estimates, was maximized because areas around known weaning dens were trapped intensely. However, the formation of the 1-day period following the independence period did not allow for re-

captures of individuals that were alive but not recaptured in the independence period. Therefore, the estimate for the independence period reflects the minimum number of individuals known alive and probably underestimates survival of juveniles.

Reproductive patterns of *D. virginiana* observed in the present study reflect the energetic demands of severe winter weather and a relatively short growing season. Females enter the breeding season in poor physical condition and actually gain weight while nursing their young. High annual mortality effectively limits each female to a single reproductive season. Under these constraints, lifetime reproductive success for most females is strongly determined by the size and survival rate of a single litter. The close, positive correlation between litter size and nutritional status of the female suggests that foraging conditions prior to and during gestation and early lactation are important. Thereafter, female behaviors that reduce mortality of juveniles, especially during the weaning and post-weaning periods when most mortality occurs, should be favored. Results from the present study indicate that, during weaning, females have certain behaviors (i.e., increased den fidelity and use of burrows in dense cover) that are likely to increase survival of juveniles during these critical periods. More research should be conducted with southern populations to determine whether these behaviors are common to the species or they have evolved under the environmental constraints imposed at the northern limit of its distribution.

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