MATERNAL TRAITS AND REPRODUCTION IN RICHARDSON'S GROUND SQUIRRELS[1]

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Abstract. Differences among conspecifics in body mass result from underlying differences in structural size and physiological condition. To determine whether the structural or physiological component of body mass has a stronger influence on reproductive traits at parturition, we studied the body composition (lean dry mass and fat content), structural size (1st principal component scores computed from 10 skeletal measurements), and body condition (residuals from regression of body mass on structural size) of yearling and older female Richardson's ground squirrels (*Spermophilus richardsonii*). At parturition, differences among yearlings in body mass primarily reflected differences in structural size associated with continuing structural growth. Older females appeared to reach a deterministic adult structural size, and body mass of older females was most strongly associated with body condition. Structural size of yearling females and body condition of older females had significant positive effects on litter mass. For older females, date of parturition had a significant negative influence on litter size. Both yearling and older females exhibited significant positive effects of parturition date and body condition on neonate mass. In older females, a trade-off resulted in smaller litters of heavier neonates as the breeding season progressed, perhaps reflecting the need for rapid growth of offspring before hibernation. Maternal size, maternal condition, and seasonal timing were important aspects of reproduction in Richardson's ground squirrels, but these characteristics are seldom considered concurrently in studies of life histories.

Key words: Alberta; body mass; body condition; litter size; neonate mass; path analysis; Spermophilus; structural size.

INTRODUCTION

Differences among conspecific animals in body mass might result from either or both of two underlying factors (Campbell and Dobson 1992, Dobson 1992). First, differences in mass could reflect differences in the supporting tissues of the body form, which can be termed structural size. Second, differences in body mass could reflect differences in the physiological or body condition of individuals, particularly as reflected by stores of energy-rich molecules such as fats. When animals are growing, body condition could be changing at the same time as structural size. For species with determinate growth of structural size, however, differences in body mass among adults might often reflect differences in body condition that are independent of differences in structural size.

Body size is thought to exert a major influence on patterns of animal life-history traits, both within (e.g., Tinkle et al. 1970, Martin 1987, Patton and Brylski 1987, Dobson 1992) and among species (e.g., Tinkle et al. 1970, Western 1979, Western and Ssemakula 1982, Stearns 1983, 1984, Dunham and Miles 1985). Although body length or a similar structural measure

was used to represent body size in some of these studies (e.g., Tinkle et al. 1970, Stearns 1984, Dunham and Miles 1985, Patton and Brylski 1987), size was represented by body mass in the other studies. Hedges (1985) suggested that body mass should be preferred as an indicator of size. Studies that used mass as a measure of size have not considered the possibility that body mass might reflect structural size and body condition to different degrees, both among individuals in populations and among species. Body mass has been equated with size in theoretical treatments of intraspecific trade-offs in life-history traits (e.g., Stearns and Koella 1986), and body mass has been applied as a structuring variable in analyses of the demographic structure of populations (Sauer and Slade 1985, 1986, 1987*a, b*). Within species, body mass can be positively associated with measures of structural size, such as linear dimensions (Iskjaer et al. 1989). The strength of this association, however, is likely to be influenced by age, season, nutrition, and reproduction (e.g., Michener 1990, Campbell and Dobson 1992, Dobson 1992).

We investigated the contributions of structural size and body condition to variation in body mass of yearling and older female Richardson's ground squirrels (*Spermophilus richardsonii*) to determine patterns of variation in aspects of body size that underlie body

mass. These ground squirrels are small (<1000 g) obligate hibernators that attain reproductive maturity as yearlings and have a single mating season shortly after they emerge from hibernation in the spring. Gestation lasts 23 d, and females typically give birth ≈27 d after emergence from a 7- to 8-month hibernation period (Michener 1985, 1989a). Yearling females, however, are not fully grown at emergence from hibernation; they emerge at 80% of the lean dry mass and total mass of older females, but achieve body mass nearly equal to older females by early lactation (Michener 1989a, 1990, Michener and Locklear 1990a, b). Because they are still growing, we expected yearlings to exhibit more individual variation in structural size and be, on average, structurally smaller than older females. We expected variation in body mass of older females to be primarily due to differences in body condition, and we expected body condition to be more variable among older females than yearling females.

Several intraspecific studies of mammals have compared maternal body mass to traits that should reflect reproductive investment at or before parturition and have found positive associations with litter size (e.g., Keller and Krebs 1970, Tamarin 1977, McClure 1981, Murie and Dobson 1987), with total mass of offspring (e.g., Clutton-Brock et al. 1982), or with both (e.g., Myers and Master 1983, Dobson and Myers 1989). These studies measured maternal body mass either near conception or just after parturition, before the massive energy investment that takes place during lactation (e.g., Kaczmarski 1966, Mattingly and McClure 1982, Kenagy et al. 1989, Michener 1989a). Maternal body mass, however, was not divided into components representing structural size and body condition, so patterns of influence of these components on reproduction were not evaluated in these studies.

For Richardson's ground squirrels, postpartum body mass of females is reportedly a significant but fairly weak predictor of the mass invested in litters at parturition (Michener 1989a). To further investigate the relationship between maternal characteristics and reproduction, we partitioned mass into structural size and body condition and considered each as possible influences on reproductive traits at birth (e.g., litter mass, litter size, and average neonatal offspring mass). We also included date of parturition as a maternal trait that might influence reproduction, because timing has been found to influence reproduction in other species (e.g., Perrins 1970, Daan et al. 1989, Dobson and Myers 1989, Hochachka 1990, Rieger 1991). In general, we expected females that were in better body condition to make greater reproductive investments. Because yearling and older females were expected to have different patterns of structural size and body condition, we also expected yearling and older females to have different patterns of influence of these traits on reproduction.

METHODS

We monitored Richardson's ground squirrels that emerged from hibernation in February and early March 1986 on an 18.5-ha pasture located 35 km north of Lethbridge, Alberta, Canada (49°52' N, 112°40' W, elevation 870 m). Because the landowner planned to eradicate this population, we attempted to remove all ground squirrels before mid-April by selectively live-trapping individuals at predetermined stages in their reproductive cycle. For this study, these stages were: recently emerged from hibernation (pre-estrous females captured within 24 h of emergence, $n = 18$), mid-pregnancy (12 d post-mating, $n = 20$), and late pregnancy (21 d post-mating, $n = 21$) for body composition analysis; and early in the last trimester of pregnancy ($n = 101$) for analysis of maternal traits and reproductive characteristics.

To achieve our goal of removing females of known reproductive status, we needed to know the dates of emergence and mating for each individual (see Michener 1985 for details). Emergence dates were determined by inspecting the site for newly emerged squirrels daily from 2 February onwards. Mating dates were determined by observing the behavior and vaginal condition of each female from emergence until mating was confirmed. Female Richardson's ground squirrels are receptive to mating for a few hours in the late afternoon, typically on their third day above ground after emergence from hibernation (Michener 1989a). Females that mate but fail to become impregnated during their first estrus become receptive a second time, about a week later.

Date of mating was determined primarily from vaginal evidence of recent insemination (Michener 1984, 1985, Michener and Locklear 1990b). Detection of a copulatory plug in the vagina or of sperm in microscopic examination of fluid from the vagina was taken as evidence that mating occurred the previous day for 110 of the 142 pregnant females. For an additional 23 females, day of mating was inferred from observation of behavior (aboveground mating or belowground consortship with one or more males) or from vaginal cytology. For the remaining 9 females, mating was assumed to have occurred 3 d after their known date of emergence from hibernation.

The 59 females used in the body composition analysis were killed immediately after final capture by being placed in an atmosphere of ether or carbon dioxide after injection of an anesthetic. Paunched mass was recorded to the nearest 0.1 g after removal of the reproductive tract and the contents of the stomach and cecum. The reproductive tract, including vagina, uterine horns, ovaries, and embryos, was weighed to the nearest 0.01 g. Paunched mass comprised three components: water (determined by oven drying at 80°C to constant mass), fat (determined by extraction of neutral lipids from duplicate 3-g samples of the ground dry

carcass with petroleum ether in a Soxhlet apparatus), and lean dry mass (calculated as the remaining mass). Lean dry mass was used as a measure of carbohydrates, proteins, and minerals found in bone, muscle, and skin.

The 101 females used in the analyses of maternal and reproductive traits were transported 3.5 km from the field site to an animal holding building where they were maintained under natural temperature and light regimes. Females were housed in plastic cages on aspen chip bedding and supplied with paper toweling for nest material. They received ad libitum amounts of rodent chow, and supplements of sunflower seeds and lettuce leaves (as a source of water) twice daily.

Of the 101 females, 97 gave birth between 24 March and 15 April, two died in captivity, and two did not deliver litters (although they had mated). As planned, most females (86/97) were captured in the last trimester of their 23 d pregnancy (2–7 d prepartum), of which the majority (92%) were captured 4–6 d prepartum. Eleven females apparently were not impregnated at first mating and remated ≈1 wk later; these females spent 9–14 d in captivity prepartum. To assess whether females had adjusted to captivity, we weighed females to the nearest 1 g before giving them their morning supplement of lettuce. Of 92 females weighed over the 2 d preceding parturition, 69 (75%) gained weight (1–23 g, average = 8.5 g, 2.7% increase in mass), one exhibited no weight change, and 22 lost weight (1–14 g, average = 5.1 g, 1.6% decrease in mass). Average weight change over the 2 d preceding parturition was similar for females that were captive ≤7 d prepartum (+5.3 g, $n = 81$) and those captive ≥9 d (+5.9 g, $n = 11$). Thus, pregnant females experienced neither large gains as a result of access to ad libitum food nor large losses as a result of capture stress, but appeared to be sufficiently adjusted to captive conditions and diet to make modest gains in mass. We included the number of days held captive in some analyses to further evaluate possible influences of captivity on reproduction.

Females were inspected for the presence of pups 5–13 times each day (average of 8.3 inspections per day). Most births (90/97 = 93%) occurred overnight, between 1700 and 0600 h. After detecting pups, we delayed handling the mother until at least 1 h elapsed without the birth of additional infants. Then, adult females were euthanized by carbon dioxide inhalation. In consultation with the Canadian Council on Animal Care, we found that neonates could be killed rapidly and humanely by inducing narcosis with carbon dioxide, then quickly cooling them at −18°C under continued exposure to carbon dioxide.

Mothers and individual neonates were weighed to the nearest 0.01 g on a Mettler balance, then the labelled specimens were stored at −20°C for later examination. The average age of litters when killed and weighed was 4.8 h (1.25–10.67 h, 76% of litters were <6 h old at weighing). Subsequent autopsy revealed that 4 of 97 females had not completed parturition at the time of euthanasia; three females had two embryos and one female had one embryo unborn even though intervals of 1.3–2.5 h had elapsed since birth of the last neonate. Because embryos weighed as much or more than the pups already born in their litter, we assumed they were healthy undelivered young, and we included them in the calculations of litter size, litter mass, and average neonate mass.

Carcasses of the 97 adult females were prepared as complete skeletons at the Provincial Museum of Alberta, Edmonton, Alberta, Canada. The following measurements were made to the nearest 0.1 mm with digital calipers: greatest length of skull, condylo-basal length, zygomatic arch breadth, ramus height, ramus length, humerus length, ulna length, femur length, tibia length, and ischium–ilium length. We designated these linear measures a priori as indicators of the size of the skeleton.

Because they had been ear-tagged as juveniles in previous years (Michener 1989b), the ages of many females (99/160 = 62%) were known, including all females 3-yr-old and older. For the remaining 61 females, age was inferred from the appearance of the nipples. A survey of known-aged females revealed that, at emergence from hibernation, the nipples of nulliparous yearlings were minute, involuted under the surrounding skin, and often difficult to locate, whereas those of older (≥2 yr) parous females were tiny, everted, and readily located. Known- and estimated-aged females were pooled for analyses.

For statistical analyses, we used t tests, the general linear model (GLM), regression (REG), analysis of covariance (ANCOVA), principal components analysis (PCA), and path-analytical techniques (STB option of the REG procedure in SAS, see also Schemske and Horvitz 1988, all procedures in SAS 1990). A single structural-size indicator was estimated as the score of each individual on the first axis (PC1) of a PCA of the covariance matrix of ten ln-transformed skeletal measurements. Body condition was estimated as the residual for each individual in a regression analysis of adult mass (post-parturition) on structural size. Dobson (1992) described the rationale for these methods.

Path analysis (Wright 1921, Li 1981, Pedhazur 1982) was used to evaluate the influences of mother's structural size, body condition, and reproductive timing on reproductive output. A path coefficient is a standardized partial regression coefficient and, therefore, represents the expected change in a dependent variable in units of its standard deviation to a change in an independent variable equal to its own standard deviation, with other independent variables held statistically invariant. Path analysis partitions the correlation between an independent and dependent variable into components reflecting the direct relationship between the variables and a degree of association due to other associations among variables. Because standardized data are

TABLE 1. Mean (± 1 SD) of body composition and reproductive parameters of yearling and older female Richardson's ground squirrels on emergence from hibernation, in mid-pregnancy (12 d post-mating) and late pregnancy (21 d post-mating). Sample sizes are 11 yearling and 7 older females for emergence, 11 yearling and 9 older females for mid-pregnancy, 12 yearling and 9 older females for late pregnancy.

	Age	Emergence	Mid-pregnancy	Late pregnancy
Lean dry mass (g)	Yearling	45.4 ± 3.1 ***	61.8 ± 2.8 *	65.8 ± 4.1
	Older	57.4 ± 3.4	65.5 ± 3.2	66.2 ± 3.8
Fat (g)	Yearling	31.8 ± 6.8	32.4 ± 7.2 *	34.8 ± 7.3 *
	Older	34.5 ± 11.5	39.8 ± 8.8	46.2 ± 14.5
Paunched mass† (g)	Yearling	190.7 ± 13.2 ***	285.0 ± 16.6 *	307.3 ± 15.9
	Older	231.2 ± 13.9	306.2 ± 23.2	317.8 ± 15.2
Reproductive tract (g)	Yearling	0.48 ± 0.12 **	5.91 ± 1.66	61.05 ± 11.59
	Older	0.76 ± 0.21	6.24 ± 2.16	52.98 ± 12.92
No. of viable embryos	Yearling		7.5 ± 1.6	7.9 ± 1.3
	Older		6.9 ± 2.0	7.3 ± 1.8

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (t test).
† Body mass after removal of the reproductive tract and the contents of the stomach and cecum.

used in path analysis, it is an especially appropriate technique for study of the effects of independent variables that are measured on different scales. Applications of path analysis to ecological systems were described by Schemske and Horvitz (1988) and Mitchell (1992).

Path analysis also requires a priori designation of plausible hypotheses of causal relationships among independent and dependent variables in a path diagram. The magnitude and significance of path coefficients provide tests of the hypotheses. We considered a high, statistically significant, path coefficient as evidence supporting the hypothesis of causal relationship between independent and dependent variables. Low, statistically insignificant path coefficients supported the null hypothesis of no direct causal relationship between variables.

RESULTS

Based on both their body (paunched) mass and their lean dry mass, yearlings were significantly smaller than older females on emergence from hibernation, but were of similar mass to older females by late pregnancy (Table 1). The lean dry mass of yearlings increased by >20 g (a 45% increase) from emergence to late pregnancy, whereas that of older females increased by <10 g (a 15% increase, Table 1). Growth in lean dry mass by yearlings did not occur at the expense of fat stores, which remained at ≈32–35 g from emergence to late gestation. Older females increased fat stores through gestation, and older females exhibited more variation among individuals than did yearlings in the magnitude of these stores by late pregnancy (F' test for equality of variances, $F' = 3.95$, $P < 0.05$). Although nulliparous yearlings had lighter reproductive tracts than older parous females on emergence from hibernation, this difference disappeared by mid-pregnancy.

Eleven of the 97 captive females gave birth ≈1 wk later than anticipated based on their estrous dates, indicating that pregnancy resulted from remating during a subsequent estrus. Because females that remated spent more time in captivity (≈12 d) before parturition than those impregnated on their first estrus (≈5 d), we examined our data set on maternal traits and reproductive characteristics for effects of the length of time in captivity. The three females aged 2 and 3 yr that were impregnated on their second estrus exhibited nearly identical reproductive characteristics to 16 similar-aged females impregnated on the first estrus (e.g., litter masses of 48.1 g and 47.0 g, respectively, $t = 0.14$, $P = 0.89$). Eight yearling females that remated, however, had significantly lighter litters than 10 yearlings that delivered litters during the same time interval but had been impregnated on their first estrus (32.2 g and 43.2 g, respectively, $t = 2.55$, $P = 0.02$). We deleted these eight yearlings from further analyses.

We compared maternal and reproductive traits of females in four age classes, from yearlings to ≥4-yr-olds (Fig. 1). Females of different ages differed significantly in postpartum body mass, and approached significance for litter mass and litter size. Females of different ages did not differ significantly in the number of days that they were held in captivity before parturition, date of parturition, or average neonate mass. Variations in traits with age were due primarily to differences between yearlings and all older females (Table 2). Females aged 2 through ≥4 years ($n = 38$) were not significantly different in postpartum body mass (GLM, $F = 2.12$, $P = 0.14$), litter mass (GLM, $F = 0.92$, $P = 0.41$), or litter size (GLM, $F = 0.60$, $P = 0.56$), whereas yearlings differed significantly in body mass, litter mass, and litter size from older females (Table 2).

Principal components analysis (PCA) was performed on the covariance matrix of ten measurements that reflected the size of the skeleton, and thus structural size. All measurements exhibited strong positive loadings on the first principal component (PC1, Table 3). In addition, PC1 could potentially explain about three quarters of the morphological variation of yearling fe-
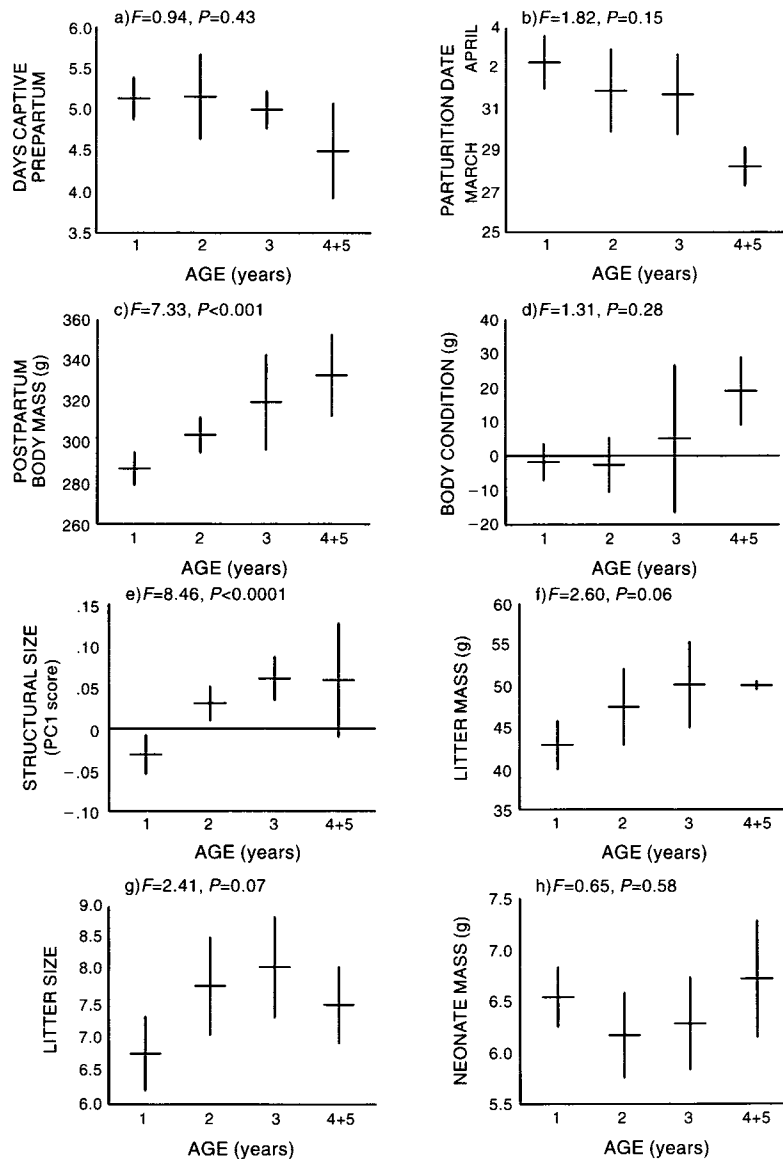
FIG. 1. Mean (± 1 SE) maternal and reproductive characteristics for female Richardson's ground squirrels of different ages. A "zero line" is presented for traits that sum to zero over all individuals. (d) Body condition is the residual score in a linear regression of postpartum body mass on structural size. Sample sizes throughout are 51 1-yr-olds, 20 2-yr-olds, 14 3-yr-olds, and 4 4- and 5-yr-olds. Probability levels from statistical tests using the GLM procedure in SAS (1990).

males, and about half of the morphological variation of older females. PC1 was clearly a "general size factor" (sensu Bookstein et al. 1985). Thus, scores of individual ground squirrels on PC1 were used as a single measure of structural size. In all subsequent analyses, a PCA was run for each statistical comparison. Analyses of covariance confirmed that slopes of within group PC1s were not significantly different among age groups, so that multiple-group PCA (Thorpe 1988) was not necessary. Structural size differed significantly among age groups (Fig. 1e), with older adult females much larger than yearlings (Table 2).

Regression of body mass on structural size was pos-

itive and significant ($R^2 = 0.50$, $F = 88.6$, df $= 1, 87$, $P = 0.0001$), and was similar for yearling and older females (ANCOVA; equality of slopes, $F = 0.10$, $P = 0.75$; equality of adjusted means, $F = 0.86$, $P = 0.36$). Body condition of each individual was estimated as the residual score (in grams) from linear regression of body mass on structural size (PC1 scores). Mean body condition of females did not differ significantly among age groups, although there was a slight and non-significant pattern of improved mean body condition that was most conspicuous in the oldest (4- and 5-yr-old) females (Fig. 1d).

For yearlings, structural size could potentially ex-

TABLE 2. Means and variances of characteristics of yearling and older female Richardson's ground squirrels at parturition. Sample sizes throughout are 51 for yearlings and 38 for older females.

	Means			Variances		
	Yearlings		Older	Yearlings		Older
Days in captivity	5.14		5.42	0.84	***	3.71
Parturition date (d)	2 April		31 March	21.11		20.22
Postpartum body mass (g)	286.99	***	313.14	837.01		897.79
Body condition† (g)	−2.13		2.86	356.27	*	708.43
Structural size‡	−3.21	***	4.30	0.73	***	0.23
Litter mass (g)	42.89	**	48.35	111.83		99.35
Litter size	6.76	**	7.76	3.98		2.62
Neonate mass (g)	6.53		6.31	1.08		0.71

* $P < 0.05$, t test or F' test; ** $P \leq 0.01$, t test; *** $P \leq 0.001$, t test or F' test.
† Body condition equals the residual score in a linear regression of postpartum body mass on structural size.
‡ Mean PC1 score and variance in PC1 score, both $\times 10^2$ (for convenience).

plain 57.7% of the variation in body mass ($F = 66.8$, df $= 1, 49$, $P \leq 0.0001$). For older ground squirrels, structural size could potentially explain only 22.0% of the variation in body mass ($F = 10.2$, df $= 1, 36$, $P = 0.003$), a significantly weaker relationship than in yearlings (comparison of R^2 terms by the F_{max} test, $P < 0.01$). In addition, yearling females were significantly more variable in structural size than older females (Table 2). Older females, however, were significantly more variable in body condition than yearling females (Table 2). Because yearling and older females differed in size, we examined the two age groups separately in further analyses.

We divided characteristics of female ground squirrels into two types. Characteristics of the females themselves were the number of days that females were held captive before parturition, parturition date, postpartum body mass, body condition, and structural size. Characteristics of the litters of females were litter mass, litter size, and average neonate mass. Postpartum body mass was strongly correlated with body condition and with structural size for both yearling and older females (Table 4). The two correlations were similar in strength for yearling females, but for older females the asso-

TABLE 3. Factor loadings on the first principal component in a PCA of structural size of female Richardson's ground squirrels.

Variable	Yearling females PC1	Older females PC1
Greatest skull length	0.22	0.24
Condylo-basal length	0.21	0.23
Zygomatic arch breadth	0.24	0.22
Ramus height	0.37	0.18
Ramus length	0.20	0.26
Femur length	0.32	0.40
Tibia length	0.41	0.41
Humerus length	0.34	0.26
Ulna length	0.38	0.41
Ischium–ilium length	0.40	0.42
% variance explained	76.2%	50.5%
Significance	≤0.001	<0.001
Sample size	51	38

ciation of body mass (BM) with body condition (BC) was significantly stronger than the association of body mass with structural size (SS) ($r_{BM,BC} - r_{BM,SS} = 0.88 - 0.47 = 0.41$, $n = 38$, $P = 0.01$).

Our interest was in possible influences of characteristics of females on the characteristics of their litters. We applied path analysis to examine characteristics of yearling and older adult females as hypotheses of causal explanation of variation in reproductive traits at birth. Our causal hypotheses invoked the possible influences of the number of days that females were held captive before parturition, date of parturition, body condition, and structural size on reproductive characteristics of females (Fig. 2). For example, the hypothesis that body condition of the mother had an effect on litter mass was tested by examination of the magnitude and significance of the appropriate path coefficient (i.e., p_3 in Fig. 2). Body condition and structural size were uncorrelated (by definition, residuals are not associated with the independent variable in a regression), and other independent variables were intercorrelated.

For yearling females, multiple regressions revealed that a significant proportion of the variation in litter mass and neonate mass could potentially be accounted for by the independent variables of days captive, parturition date, body condition, and structural size (litter mass $R^2 = 0.22$, $F = 3.23$, df $= 4, 46$, $P < 0.05$; neonate mass $R^2 = 0.19$, $F = 2.77$, $P < 0.05$), but litter size was not significantly explained by the independent variables ($R^2 = 0.15$, $F = 2.00$, $P = 0.11$). For ≥2-yr-old females, the variation in reproductive characteristics that could potentially be explained by the independent variables was somewhat higher (litter mass $R^2 = 0.37$, $F = 4.90$, df $= 4, 33$, $P = 0.003$; litter size $R^2 = 0.33$, $F = 4.02$, $P < 0.01$; neonate mass $R^2 = 0.49$, $F = 7.87$, $P \leq 0.0001$). For each of the path diagrams in Fig. 3, the path coefficient for the unexplained variance of the path analysis (p_5 in Fig. 2) is $\sqrt{1 - R^2}$ for each of the R^2 terms.

For yearling females, there were significant positive path coefficients from structural size to litter mass, from structural size to litter size (the R^2 for this path

TABLE 4. Correlations among characteristics of yearling (above the diagonal) and older (below the diagonal) female Richardson's ground squirrels and their litters at parturition. Sample sizes throughout are 51 for yearlings and 38 for older females.

	Days captive	Parturition date	Body mass	Body condition	Structural size	Litter mass	Litter size	Neonate mass
Days captive		−.00	.09	.02	.09	−.01	−.10	.21
Parturition date	.56***		−.39**	−.17	−.36**	−.11	−.19	.24
Body mass	.06	.00		.65†	.76†	.41**	.23	.15
Body condition	.08	.11	.88†		.00	.10	−.05	.26
Structural size	−.01	−.20	.47**	.00		.45***	.35**	−.02
Litter mass	−.22	−.28	.50***	.49**	.15		.86†	−.24
Litter size	−.31	−.53***	.21	.11	.23	.79†		−.67†
Neonate mass	.24	.45**	.47**	.58***	−.08	.22	−.40**	

** $P \leq 0.01$ (there was no P between 0.01 and 0.05).
*** $P \leq 0.001$.
  † $P \leq 0.0001$.

diagram, however, was not significant), and from parturition date and body condition to average neonate mass (Fig. 3). For older females, there were significant positive path coefficients from body condition to litter mass and average neonate mass, a negative path coefficient from parturition date to litter size, and a positive path coefficient from parturition date to average neonate mass (Fig. 3). All other potential effects of female characteristics on reproductive characteristics were not significant.

For 33 of the older females, body mass at the time of mating in the field was known. We examined patterns of reproduction and maternal mass within 2 d after estrus (=mating), in order to evaluate the basis of changes in condition of mothers. Regression of body mass at estrus on structural size was positive and significant ($R^2 = 0.42$, $F = 22.4$, df $= 1, 31$, $P < 0.0001$). We used the residuals of this analysis as an index of body condition at estrus. We substituted this condition index at estrus into path analyses of litter mass and average neonate mass (Fig. 2), but neither regression was significant ($R^2 = 0.21$, $F = 1.9$, df $= 4, 28$, $P =$

FIG. 2. The path diagram for the effects of maternal characteristics on reproduction in Richardson's ground squirrels. r_1–r_5 are correlations among independent variables. The reproductive trait (e.g., litter mass) is the dependent variable. p_1–p_4 are path coefficients of the effect of the independent variables on the dependent variable. U is the "unexplained" residual variance (the variance in the dependent variable that could not be accounted for statistically by the independent variables), and p_5 is the path coefficient of the residual variance.

0.14; $R^2 = 0.17$, $F = 1.5$, $P = 0.24$; respectively). Furthermore, paths from body condition at estrus to litter mass and average neonate mass were not significant ($p = 0.29$, $P = 0.10$; and $p = -0.06$, $P = 0.74$; respectively). The major patterns revealed in the path analyses of reproduction of older females based on postpartum mass (Fig. 3) were not evident for ≥ 2-yr-old females at estrus.

Assuming that the 33 females ≥ 2 yr old were fully grown in structural size, then the gain in mother's mass between estrus and post-parturition might represent maternal investment in personal body condition during gestation. Because the gestational gain in maternal mass was not positively or significantly associated with structural size ($r = -0.17$, $n = 33$, $P = 0.35$), this gestational gain was substituted directly into path analyses for body condition (Fig. 2, but allowing correlation of body condition and structural size). Multiple regressions for litter mass, litter size, and average neonatal mass were all significant ($R^2 = 0.28$, $F = 2.7$, df $= 4, 28$, $P = 0.05$; $R^2 = 0.31$, $F = 3.1$, $P = 0.03$; $R^2 = 0.45$, $F = 5.8$, $P = 0.002$; respectively). The significant paths based on gestational gain in maternal mass were the same as those obtained using postpartum mass (Fig. 3). Path coefficients were: 0.40 from body condition to litter mass ($P = 0.03$), -0.38 from parturition date to litter size ($P = 0.04$), 0.36 from parturition date to neonate mass ($P = 0.03$), and 0.55 from body condition to neonate mass ($P = 0.001$).
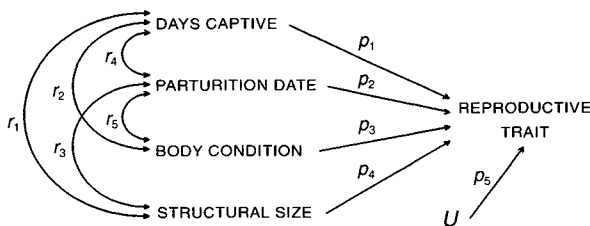
DISCUSSION

We assumed that, for adult female Richardson's ground squirrels, body condition was adequately reflected by our index of residuals of postpartum mass regressed on structural size. This assumption appeared to be justified from two lines of indirect evidence. First, because there was no apparent strong influence of body condition at estrus on reproduction of older mothers, we could use the difference between estrous mass and postpartum mass as an alternative condition index. This alternative index based on gestational gain in maternal

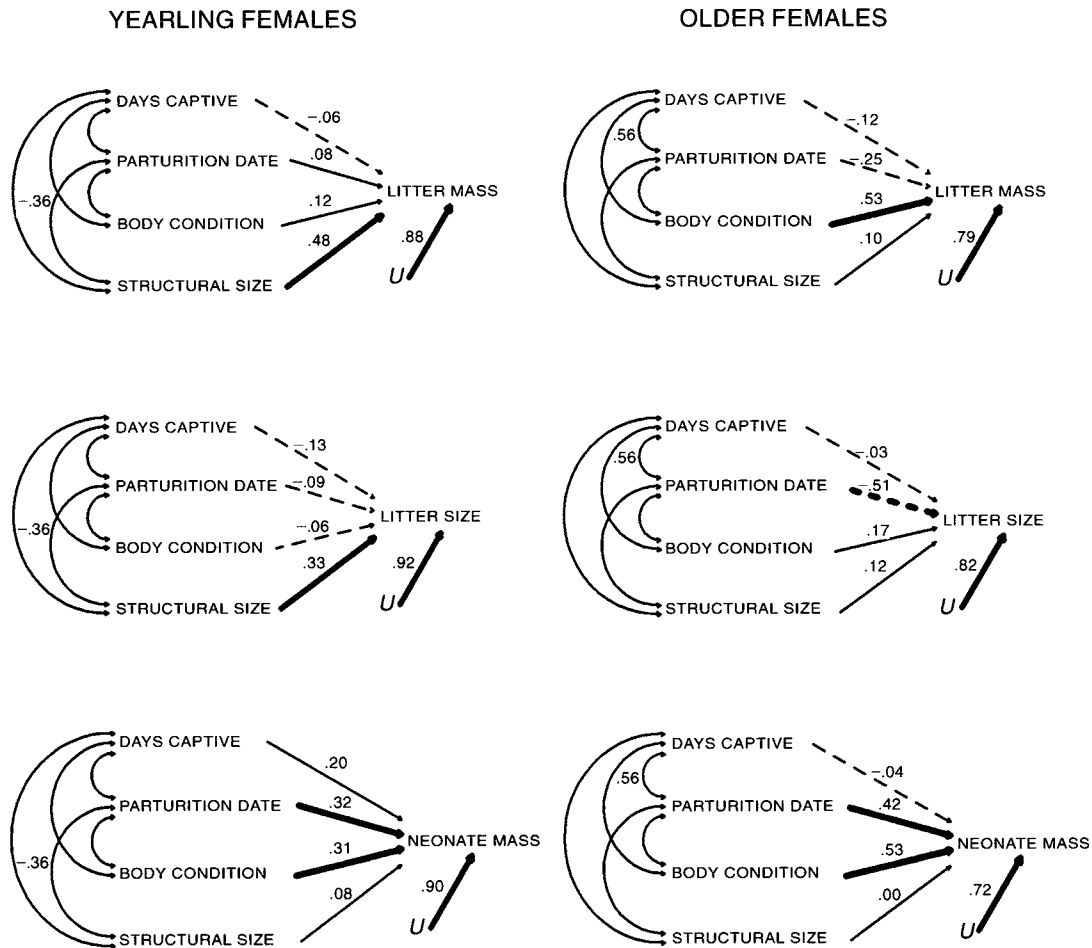YEARLING FEMALES   OLDER FEMALES

FIG. 3.   Path diagrams, after Fig. 2, of the effect of maternal characteristics on reproductive traits for yearling and older female Richardson's ground squirrels. Only significant correlations are indicated among the independent variables. Paths with significant ($P < 0.05$) path coefficients are indicated by thick lines. Positive paths are represented by solid lines and negative paths by dashed lines. Sample sizes are 51 yearlings and 38 older females.

mass exhibited the same pattern of influences on reproduction as our index based on regression residuals derived from postpartum mass. The alternative index would, however, be an inappropriate index of condition for yearling mothers because their gestational increase in mass included growth in structural size. Second, variances of both fat content in late pregnancy and our regression-based body condition index were significantly greater for older than yearling females (Tables 1 and 2), as we would expect if these two measures reflected the same underlying property.

Females of different ages differed in stage of growth at emergence from hibernation. Although yearling female Richardson's ground squirrels were reproductively mature, they were not fully grown when they emerged from hibernation (see also Michener and Locklear 1990a, b). Lean dry mass (reflecting structural elements of size from bone, muscle, and skin) increased substantially for yearling females be-

tween spring emergence from hibernation and late pregnancy (Table 1).

Post-hibernation skeletal growth could not be measured directly in the larger sample of postpartum females, but yearlings averaged significantly smaller in structural size than older females (Table 2). Thus, two methods of assessing structural body size suggested that yearling females were growing during gestation, and we anticipated that yearlings and older females would exhibit different patterns of association of body mass, structural size, and body condition at parturition.

Postpartum body mass of yearling females reflected structural size and associated body condition, and both size and condition were strongly correlated with body mass. In contrast, in older females body mass primarily reflected body condition, and these variables covaried to a much greater extent than body mass and structural size. Individual yearling female ground squirrels might

have reached somewhat different stages of growth in structural size by the time of parturition, as indicated by the significantly greater variance in structural size of yearlings compared to older females. The structural size of older female ground squirrels appeared to plateau (Fig. 1e), indicating that 2-yr-old females were at or near their full structural size. The decreased correlation between structural size and body mass noted for female ground squirrels as they aged (Table 4) also occurs in meadow voles (*Microtus pennsylvanicus*; Campbell and Dobson 1992).

Structurally larger yearling mothers had heavier litters than smaller yearlings, primarily through a positive influence of maternal size on litter size (Fig. 3). If different structural sizes among yearlings partially reflected degree of growth of structural body size, then degree of investment in reproduction may have been associated with developmental stage. Yearling mothers in good body condition had heavier neonates than those in poorer condition, and yearlings that gave birth later in the season produced heavier neonates. However, correlations between body condition and neonate mass and between parturition date and neonate mass were not significant (Table 4). This somewhat surprising result obtains because path analysis examines relationships between pairs of variables with other correlations held statistically invariant. Apparently, the influence of other correlated independent variables obscured the strength of the relationship between body condition and neonate mass and between parturition date and neonate mass. The additional correlations apparently did not have to be highly significant to produce an obscuring effect. Thus, path analysis was likely better than correlation for identifying relationships between maternal and reproductive traits.

Our interpretation of potential influences of maternal traits on reproduction in older female Richardson's ground squirrels (Fig. 3) was that body condition and parturition date significantly affected reproduction, but structural size did not. Older mothers in better body condition had heavier litters and heavier individual neonates than lighter mothers. Mothers that emerged from hibernation later had smaller litters but heavier neonates than early-emerging females, and this trade-off resulted in the total mass invested in litters being little affected by parturition date. In Uinta ground squirrels (*S. armatus*), Rieger (1991) found a pattern of later litters being smaller but having heavier offspring at weaning. This trade-off eventually resulted in juveniles from early and late litters entering hibernation together at a similar mean body mass. Early offspring have the advantage of a longer period of time for growth before hibernation must occur. As the breeding season progressed, Uinta ground squirrel mothers at least partly compensated for the shorter growth season before hibernation by weaning smaller numbers of heavier offspring. Our results indicate a similar pattern in Richardson's ground squirrels at parturition.

Rowe et al. (1994) presented a model to explain the seasonal decline in clutch size commonly exhibited within bird populations, and suggested that the model could be applied to other taxa. Female Richardson's ground squirrels ≥2 yr old exhibited a strong seasonal decline in litter size (Table 4). Rowe et al.'s (1994) model invokes a trade-off between the benefit of delaying reproduction while body condition improves and the loss in value (fitness) of offspring produced late in the season. Our results and Rieger's (1991) suggest that mothers may compensate young produced later in the breeding season with increased energy (mass) investment, so that early and late young have similar value at the end of the annual activity season. This compensation requires that litter size decline as the breeding season progresses, and provides an alternative trade-off hypothesis to test against the Rowe et al. (1994) model.

The mechanism of the trade-off between number and quality of offspring of older females involves a complex pattern of maternal investment in the litter and preparation for the considerable energetic requirements of lactation. Mothers gain personal mass during gestation (Table 1), as well as investing in their litters. Individuals that make greater investments in the mass of their litters also exhibit greater body condition at parturition (Fig. 3, Table 4). This pattern of co-investment in the older mother's litter and body condition occurs during gestation and is not carried over from the previous year, as evidenced by the lack of association of the older mothers' body condition at estrus and subsequent reproduction.

Additional insights into the influence of maternal traits on reproduction can be gleaned from the path analyses. For mothers in different age classes, different maternal characteristics influenced reproductive investments. For yearlings, structural size influenced litter mass through an influence on litter size, whereas in older females, body condition influenced litter mass through an influence on neonate mass. Age-dependent differences in the influence of maternal characteristics on reproduction at parturition corresponded to differences in growth trajectories during gestation. Yearlings exhibited a substantially greater increase in lean dry mass during pregnancy than older females (Table 1). Another maternal characteristic that exhibited age-dependent differences was the relationship between parturition date and litter size. For older females, litter size decreased significantly as the breeding season progressed, a pattern that yearlings did not share.

Path analysis also revealed some consistent patterns between yearling and older females. The path model could explain significant amounts of variation in neonate mass, ≈20% for yearlings and ≈50% for older females. For both age classes, mothers in better body condition had heavier offspring at parturition, and females that gave birth later in the season had heavier offspring (Fig. 3). Thus, the compensation of greater

investment in individual offspring that are produced later in the season may be a general phenomenon of both growing and fully grown mothers. Multiple regression indicated that maternal characteristics had a stronger potential influence on the mass of neonatal offspring than on litter size, and a much stronger effect in older than in yearling females. This result suggests that, for Richardson's ground squirrels, the quality of offspring may be an equally or more important aspect of reproduction as the number of offspring.

Body mass clearly reflected different aspects of body size in yearling and older female Richardson's ground squirrels. Also, despite some similarities, reproduction appeared to be influenced in different ways by the two aspects of body size, depending on the stage of growth of the mother. These conclusions could not be discerned without partitioning body mass into structural size and body condition. Thus, for many species, the meaning of intraspecific associations of maternal body mass and reproductive characteristics that are widely reported in the literature (e.g., Sauer and Slade 1987*b* and references therein) cannot be clearly evaluated until further research is completed, particularly if females begin reproduction before they are fully grown. Interspecific studies that span several orders of magnitude of body mass, however, might accurately reflect associations of structural size and reproductive characteristics.

Another important conclusion of our study was that for a small mammal, seasonal timing (parturition date) had substantial influences on the number and quality of offspring (Fig. 3). In particular, ≥2-yr-old females that reproduced earlier in the breeding season had significantly larger litters. Seasonal timing of reproduction is strongly associated with reproduction in many bird species (e.g., Klomp 1970, Drent and Daan 1980, Daan et al. 1989, Hochachka 1990, Hepp and Kennamer 1993, Rowe et al. 1994, but see Martin and Hannon 1987). Studies of similar phenomena for mammals are generally lacking, although a seasonal decline in litter size has been studied for meadow voles (Dobson and Myers 1989), a species in which individual mothers may have more than one litter during a breeding season (Keller 1985). Seasonal timing of reproduction needs to be included in future studies of mammalian life histories.

Multiple regressions revealed that most of the variation in reproductive characteristics of female Richardson's ground squirrels remains to be explained. Only 15–22% of the variation in litter mass, litter size, and average neonate mass of yearling mothers and 33–49% of the variation of older mothers could be potentially explained by maternal traits. Thus, the majority of the variation in reproduction of the ground squirrels must be accounted for by variation in other variables, such as the mother's genotype or the ecological environment. Even so, the present study demonstrates the importance of examining structural size and body condition separately, and including seasonal timing of reproduction, when studying the influence of maternal traits on reproduction.

Non-destructive measurements of structural size and body condition are needed for further research. Bailey (1968) and Chapman et al. (1977) estimated body condition of cottontail rabbits (*Sylvilagus floridanus*) as the ratio of body mass to the cube of total body length. Schluter and Gustafsson (1993) used tarsus length, measured externally on collared flycatchers (*Ficedula albicollis*), as an indicator of structural size, and they estimated body condition from the residuals in a regression of body mass on tarsus length. External measurements of the body, however, may not reflect structural size as well as skeletal measurements. Refinement of x-ray and electrical conductivity technology (e.g., Scott et al. 1991, Campbell and Dobson 1992) may facilitate accurate measurements of the skull and skeleton and determination of fat content for vertebrate animals living in the wild.

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