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AGE VARIATION IN VOLES *(MICROTUS CALIFORNICUS, M. OCHROGASTER)* AND ITS SIGNIFICANCE FOR SYSTEMATIC STUDIES

By

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Age variation plays an important role in systematic studies. This is especially true for arvicolid (= microtine) rodents, most of which do not have a definitive adult size. Problems arise when comparing samples from different localities and unknown, but probably different, age structure. Many authors assume that age variation is the same in the several populations analyzed. There is no way to test whether the observed differences in morphology are due to geographical, environmental or age variation, unless some of the factors influencing variation are known or can be estimated. The purposes of this paper are to analyze the nature of ontological variation in morphology during the course of post-natal growth in known-age voles of two different species in order to determine: 1) which characters may be measured more reliably; 2) whether significant interspecific differences in growth patterns and morphology occur; 3) which characters best discriminate between species; and 4) which characters are least influenced by age, and which are most influenced, in order to predict age from skull morphology.

Chitty (1952) was the first to note that season of birth influenced subsequent growth rate in juvenile *M. agrestis;* young born in spring or early summer grew rapidly, and attained puberty during the summer of their birth, whereas young born in late summer or fall grew slowly, if at all, until the following March. This pattern was subsequently confirmed by Cowan and Arsenault (1954) for *M. oregoni.* Barbehenn (1955) also found differential growth and size in *Microtus pennsylvanicus;* males born

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after mid-June did not reach puberty in the same season, while it took females born at the same time six weeks to reach that stage. Differences could not be related to soil factors, weather or forage composition. Bee and Hall (1956) noted that in *Microtus miurus*, individuals born in the winter grow more slowly and never become as large as individuals born in the spring. Pinter (1968) found that body weight in *Microtus montanus* was positively correlated with day-length and amount of food. Martinet and Spitz (1971) pointed out the influence of photoperiod and quality of food on growth in *Microtus arvalis*, and Pistole and Cranford (1982) recorded reduced growth rate in *M. pennsylvanicus* under short photoperiod. Pokrovski (1971) noted that for *Lagurus lagurus* and *Microtus gregalis* average age of initial reproductive activity depended on date of birth, and that there is seasonal variation in body weight, which differs in successive generations. In *Microtus oeconomus* there is furthermore a significant difference in weight of crystalline lens in specimens of the same age born in spring or born at the end of summer.

Lidicker (1973) found that the period of reduced or suspended growth in *M. californicus* was not winter in the Mediterranean climate of coastal California, but rather was during the dry season, usually June through October, under field conditions. Brown (1973) studied *Microtus pennsylvanicus* in the field and reported seasonal differences in growth. Young born in spring and early summer reached adult size in twelve weeks or less, and then lost weight in fall. Animals born in middle to late summer stopped growing in the fall and resumed growth in the spring; they maintained weight throughout the Minnesota winter. In contrast, Iverson and Turner (1974), studying the same species under the more severe winter conditions typical of Manitoba, found that individuals lost considerable weight during mid-winter before beginning to gain again in February. Winter weight reduction in juveniles was also found in *M. xanthognathus* in central Alaska by Wolff and Lidicker (1980), who interpreted the phenomenon as a means of reducing food requirements. Thomas (1976) found that in several rodents, craniometric variation was correlated with climatic variables such as length of growing season, precipitation, temperature, moisture deficit and evapotranspiration. Daketse and Martinet (1977) noted for *Microtus arvalis* a decrease in body growth and fertility with increasing temperature. Largest and most fertile animals were those raised at low temperatures, under long-day conditions and fed with alfalfa harvested in the spring. Huminski and Krajewski (1977) found a higher body growth rate during a warm winter than in a cold one for *Microtus arvalis*. Voles kept in the laboratory showed the least inhibition of growth. Cole and Batzli (1978) noted an influence of supplemental food on body growth of *Microtus ochrogaster*, and Batzli *et al.* (1977) found that growth could also be suppressed by social factors. Kaneko (1978) also observed seasonal and sexual differences in absolute and relative growth for *Microtus montebelli*, and Tast (1978) reported variation from year to year in weights of over-wintering *M. oeconomus*. Inhibition of juvenile growth rate because of progeny-adult social interactions was first sug-

gested for *M. townsendii* by Boonstra (1978), and similar results were obtained by Smolen and Keller (1979) for *M. montanus*.

Petterborg (1978) showed that the length of photoperiod affected body weight in *Microtus montanus*. Animals raised under a longer photoperiod gained weight more rapidly than those raised under a short one. Thyroxin levels were correlated with length of photoperiod.

Figure 1 summarizes these observations and also includes additional factors which may play a role in growth, *e.g.* behavior and competition. Some of the factors interact. Temperature and moisture are interdependent; their levels can have an influence *per se*, but a seasonal cycle can be superimposed on them with effects on the vegetation, *i.e.* food resources. Sampling techniques, by selecting animals of given sex, age or hierarchical position in the population, can also be a source of bias in systematic investigations (Pizzimenti, 1979).

In systematics, phenotype is used to infer genotypic relationships between individuals (Fig. 1). As pointed out by Frelin and Vuilleumier (1979), a certain amount of information is lost or undergoes transformation in the ontogeny of an individual. Many factors alter the expression of the genotype and one has to be aware that the skull of a vole, for instance, expresses only part of the genetic information. As long as it is possible to estimate the importance of the different components of variation, we are able to make meaningful comparisons, systematically speaking, between individuals or populations. Only then are we sure to compare similar components of variation. As an example, the following questions can be asked: are the differences observed when comparing animals from two or

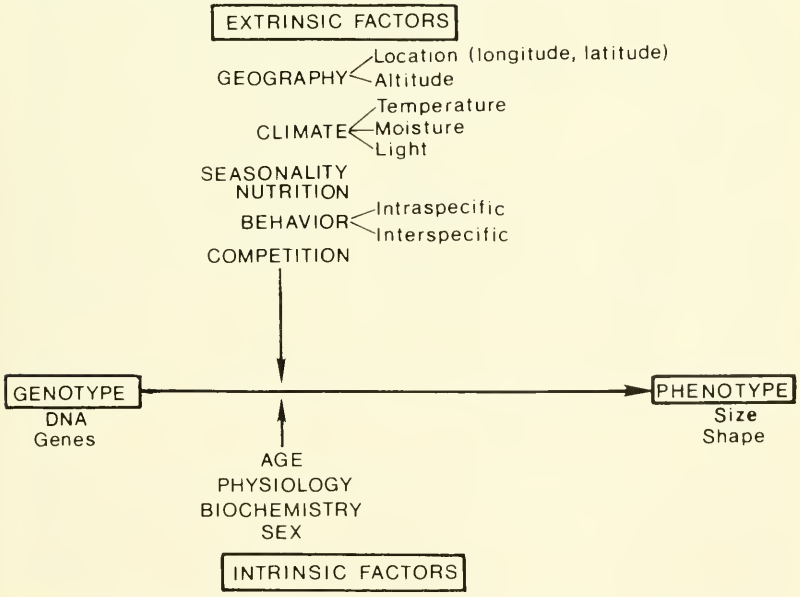

FIGURE 1.—Diagram of factors influencing growth and development in arvicolid rodents.

more localities only geographical and not due to age, food, or seasonality? Anderson (1959) discussed this problem and pointed out the different sources of variation which can overshadow or be mistaken for geographic variation. Most authors who studied variation (age, geographic) in arvicolids (Howell, 1924; Goin, 1943; Stombaugh, 1953; Snyder, 1954; Martin, 1956; Anderson, 1956, 1959, 1960; Choate and Williams, 1978) used morphological features such as degree of development of lambdoidal crests, frontal ridges or sutures to assign animals to given age classes, thus reducing the influence of age variation. However, the descriptions of characters given are usually imprecise, resulting in groups lacking homogeneity. Frank and Zimmermann (1957) considered that for *Microtus arvalis* "variability of growth is so important in all age classes, that age determination, based on morphological characters is impossible." A recent systematic study of *Pitymys* (Spitz, 1978) does not even mention age variation and includes all the specimens collected. Zejda (1971), working with *Clethrionomys glareolus*, observed that in systematic studies, animals of the same developmental stage, even though of different ages, should be used. For example, overwintered individuals captured in June or later, while representing a mixture of different cohorts, have one characteristic in common: their growth is almost complete. Zejda's approach would be feasible if there were only a delay in growth; *i.e.* if all specimens eventually reached a given size after a certain lapse of time. Unfortunately this is not the case.

Thus, skulls exhibiting similar morphological features may not be the same age, while skulls of the same age are not necessarily morphologically identical, even when growth is completed. Since seasonal variation is important in voles, it is advisable to select animals collected at the same time, and of those, choose specimens of the same age (see Anderson, 1959). An obvious drawback of this procedure is that it often reduces sample size to a point which makes modern statistical methods inapplicable.

One of the purposes of the present paper is to study age variation in *Microtus californicus* and *M. ochrogaster* and predict age on the basis of skull measurements. Similar studies have been done by Lidicker and MacLean (1969) on *Microtus californicus* and by Hoffmeister and Getz (1968) on *Microtus ochrogaster*, employing voles reared in captivity. The material used here is, in part, the same used in those previous studies. The influence of age structure in samples when comparing species and sexes with each other was also investigated. Furthermore, some relationships of size and shape have been examined using principal components and canonical correlation analyses.

Microtus californicus and *M. ochrogaster* have allopatric distributions (Hall, 1981), and are placed in two different subgenera, *Microtus* and *Pedomys*. There are differences in the bacula (Anderson, 1960). However, the anatomy of the diastemal palate (Quay, 1954a) and the Meibomian glands (Quay, 1954b) are not greatly different and the chromosome numbers are the same in both species (Matthey, 1957).

The main sources of variation (specific, age, sexual) are known. They are also of different orders of magnitude. This should enable us to interpret our results with fewer difficulties than when dealing with groups in which many factors can be responsible for the observed variation.

MATERIALS AND METHODS

Specimens

Of the 373 specimens of lab-reared *Microtus californicus* used by Lidicker and MacLean (1969) we used 314. Those eliminated were either older than one year or showed malformations which made measurements unreliable. Only 144 specimens from the original 191 studied by Hoffmeister and Getz (1968) were used for that reason, and also because some young individuals had skulls too fragile to measure. Twenty specimens of *Microtus ochrogaster* from the field (8 males and 12 females) of known age, collected by Martin (1956) were added to those from Illinois, increasing the sample size to 164. Table 1 summarizes the sample sizes according to age and sex of the two species considered.

Measurements

A total of 48 skull measurements (to the nearest 0.1 mm), plus mandibular and cranial weights (to the nearest mg) were taken for each

TABLE 1. Sample sizes by species, sex, and age class (field coll.).

AGE (in days)	*M. californicus*		*M. ochrogaster*	
	males	females	males	females
0- 19	21	13	4	3
20- 39	14	12	7	9
40- 59	28	22	8 (1)	5 (2)
60- 79	17	15	10 (3)	4 (2)
Age-Class 1	80	62	29 (4)	21 (4)
80- 99	20	19	5 (1)	8 (1)
100-119	23	19	1 (1)	2 (2)
120-139	12	15	8 (2)	5 (3)
140-159	15	4	8	2
160-179	13	9	0	0
180-199	3	6	7	5 (1)
Age-Class 2	86	72	29 (4)	22 (7)
200-219	0	1	4	10
220-239	1	1	0	0
240-259	1	2	4	6
260-279	1	0	8	2
280-299	2	2	0	0
300-319	0	0	3	8
320-339	0	0	7	2
340-359	1	1	0	0
360-379	1	0	4	5 (1)
Age-Class 3	7	7	30	33 (1)
Total	173	141	88 (8)	76 (12)

specimen. Head and body lengths were obtained from specimen labels. The measurements are described in Table 2, and illustrated in Figs. 2 and 3. Measurements were divided into 3 groups: lengths (L), widths or breadths (B) and heights (H). Those marked by * were taken with needle point calipers, those by a + with an occular micrometer (10×); all others were taken with dial calipers. For H1, a glass blade was put underneath the bullae tympanicae, thus defining a plane passing through the last upper molars, from which we measured the distance to the top of the skull. Thickness of the blade was then subtracted. The skulls were weighed on a Mettler balance.

TABLE 2. Descriptions of cranial and mandibular measurements used. * measured with needle-point calipers. + measured with ocular micrometer. All other measurements conventional calipers.

L1	condylo-incisor length	posterior point of occipital condyle to most anterior part of incisor
L2	condylo-incisive length	posterior point of occipital condyle to anterior point of incisor at its alveolus (1)
L3	occipito-nasal length	posterior point of occipital bulge to anterior point of nasal (1)
L4	condylo-zygomatic length	posterior point of occipital condyle to antero-superior edge of zygomatic process of maxilla (2)
L5	alveolo-incisor length	posterior end of last molar to anterior point of incisor at its alveolus (2)
*L6	diastema length	anterior point of alveolar margin of 1st molar to posterior point of alveolar margin of incisor (3)
*L7	alveolar length of upper molar toothrow	posterior point of alveolar margin of last molar to anterior point of alveolar margin of first molar (1)
L8	upper molar toothrow length	measured at the crowns
L9	length of incisive foramen	anterior to posterior point of foramen (1)
*L10	incisivo-foramen length	anterior point of incisive foramen to posterior point of alveolar margin of incisor
L11	hamular-toothrow length	anterior crown of 1st upper molar to posterior part of hamular process
L12	condylo-molar length	posterior point of occipital condyle to anterior crown of 1st upper molar
L13	nasal length	anterior point of nasal to suture with frontal (1)
*L14	frontal length	naso-frontal suture to fronto-parietal suture in the sagittal plane
*L15	parietal length	fronto-parietal suture to parieto-interparietal suture in the sagittal plane
*L16	interparietal length	parieto-interparietal suture to interparieto-supraoccipital suture in the sagittal plane
L17	zygomatic aperture length	anterior to posterior margin of zygomatic aperture (1)
+L18	basioccipital length	basioccipito-basisphenoid suture to closest point on margin of foramen magnum in the sagittal plane
L19	mandibular toothrow length	measured at the crowns
L20	mandibular length 1	most anterior part between coronoid process and condyle to anterior (= lowest) point of incisor at its alveolus

TABLE 2. (Continued)

*L21	mandibular diastema length	anterior point of alveolar margin of 1st lower molar to posterior point of alveolar margin of incisor (see Fig. 3A)
L22	mandibular length II	most posterior part of condyle to most anterior part of 1st molar at the crown
L23	supraoccipital length	upper margin of foramen magnum to interparieto-supraoccipital suture in the sagittal plane
L24	supraoccipital-interparietal length	upper margin of foramen magnum to parieto-interparietal suture in the saggital plane
L25	mandibular length III	most posterior part of condyle to anterior (= lowest) point of incisor at its alveolus
*L26	alveolar length of mandibular toothrow	as for L7
B1	nasalia width	greatest width over nasalia
B2	rostral width	across bulge over foramen infraorbitale (3)
B3	zygomatic width	between the lateralmost points on zygomatic arch (1)
B4	interorbital width	between medial points of interorbital constriction (1)
B5	lambdoidal width	across lambdoidal processes (greatest width) (2)
+B6	incisive foramen width	greatest width
+B7	palate width	between medialmost points on alveolar margin of 1st molar (1)
+B8	pterygoid width	between most anterior margin of pterygoid fossae (see Fig. 3B)
+B9	hamular width	greatest width across hamular processes
B10	paroccipital width	greatest width across paroccipital processes
B11	condylar width	greatest width across external margin of occipital condyle
B12	foramen magnum width	greatest width of foramen magnum
B13	incisor width	greatest width at level of anterior alveolus of incisors (see Fig. 3C) (4)
B14	anteorbital constriction width	between medianmost part of fossae
H1	skull height I	perpendicular distance from a plane going through the most inferior part of the bullae along the crown of the most prominent molar, to highest point on cranium (2)
H2	skull height II	from basioccipito-basisphenoid suture to interparieto-parietal suture in the sagittal plane
H3	skull height III	from anterior alveolar margin of 1st molar to suture between nasal and frontal
H4	zygomatic arch height	greatest height (usually near maxillar-jugal suture)
H5	foramen magnum height	greatest height
H6	mandibular height I	from lowest point on angular process to highest point on condyle
H7	mandibular height II	from lowest point between coronoid process and condyle to closest point on anterior part of angular process
H8	mandibular height III	from anterior alveolar margin of 1st molar to posterior face of symphyseal eminence (see Fig. 3D)

HB	head and body length	(1) after Anderson (1969)
CRANW	cranial weight	(2) after Howell (1924)
MANDW	mandibular weight	(3) after Pietsch (1970)
		(4) after Lidicker and MacLean (1969)

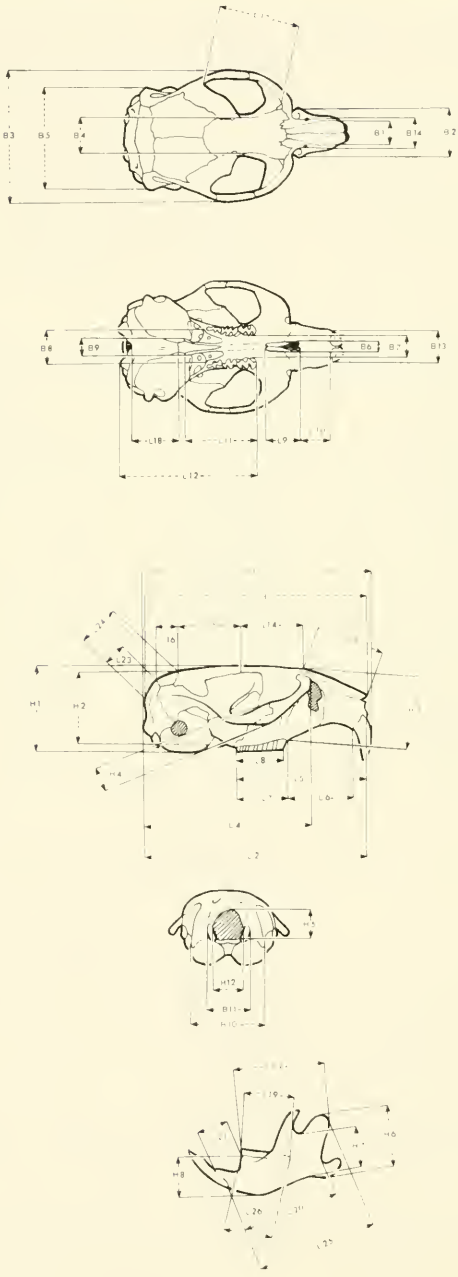

FIGURE 2.—Diagram of cranial and mandibular measurements employed in this study.

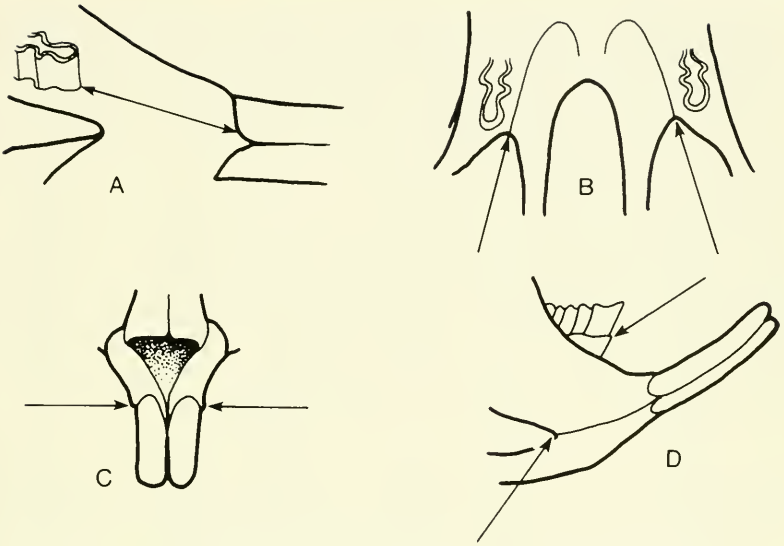

FIGURE 3.—Detail of certain measurements employed in this study; see also Table 2.

Computations and programs used

Computations were made on the Honeywell 66-60 computer of the University of Kansas Academic Computer Center. The following BMDP programs were used (Dixon and Brown, 1977): P-AM, description and estimation of missing data; P-2R, stepwise regression; P-9R, all possible (best) subsets regression; P-4M, factor analysis (principal components option); P-6M, canonical correlation analysis; and P-7M, stepwise discriminant analysis.

Estimation of missing values

Some measurements could not be taken on certain specimens, resulting in data matrices with missing values. Because programs used in analyses delete cases which have missing values in one or more variables, we estimated missing values with the program which uses simple regression on variables that showed the greatest correlation with the missing variables. Many skulls were damaged in the rostral region, so measurement B2 was eliminated from multivariate analyses. For the remaining variables, 37 (22.6%) of the *M. ochrogaster* specimens had missing values, while in *M. californicus* there were 27 (8.6%). Overall, there were 314 missing values distributed over 64 specimens.

RESULTS

Accuracy of measurements

Ten specimens (five males and five females) were measured repeatedly to estimate measuring error (seven of them five times, two six times and one 10 times). The specimen measured 10 times was included twice in

each series, first as specimen 1, and then as specimen 5.

A two-way ANOVA, for which we considered only the first 5 measurements for each specimen, made it possible to determine whether the results differed significantly from each other. Only L13 and H2 showed significant values for F_C (test between the columns) because they are difficult to define accurately. L5, B11, B12, and H1 also exhibited rather high F_C values.

Table 3a presents means, standard deviations, coefficients of variation (CV) and standard errors for the 10 repeated measurements for all skull variables of KU 84940. The relative error made in measuring a given dimension is given by the CV values (Sokal and Rohlf, 1969). L15, L16, B6, B7, B13, H4 show high values. Table 3b gives a weighted average for the standard deviations of all specimens measured repeatedly and computed according to the formula,

$$\bar{s} = \sqrt{\frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2 \ldots \Sigma(x_i - \bar{x}_i)}{n_1 + n_2 \ldots + n_i - i}}$$

where $\bar{s}$ for each measurement is the square root of the sum of the sums of deviation squares for each individual i divided by the corresponding number of degrees of freedom (n = no. measurements). In cases with skull damage, only 9 (8 or 7) specimens were used, because a given measurement could not be taken. In Figure 4, a mean $\overline{CV}$, computed using $\bar{s}$ and a mean $\bar{x}$ for the 10 measurements, was plotted against $\bar{x}$ for each variable and according to the measuring technique. The curve of expected CV was computed by assuming for all of the variables, an $\bar{s}$ equal to 0.0496, the value obtained for L1. That allowed comparisons of the different measurements with each other. The CV-values of Figure 4 agree quite well with those of Table 3a for specimen No. 84940. There are a few exceptions, however; L17, which is more variable in No. 84940, and L18 with the opposite tendency. By examining Figure 4, it is possible to select among the plotted variables those which are close to the curve or even below it. They are the ones less subject to measuring error, hence, the most reliable. No obvious correlation with the measuring technique used is apparent. Quite a few width measurements lie below the curve. Unfortunately, some of the variables showing a relatively great amount of measuring error are precisely those particularly interesting for systematic studies or age estimation; this is discussed below.

Principal components analysis (PC-analysis)

Principal components were extracted on the correlation matrix of $\log_{10}$ transformed variables. The PC-analysis performed on the whole sample (n = 478) revealed that approximately 65% of the total variation was explained by the first factor (Table 4). A plot of the first two PCs showed the species well separated (Fig. 5). Younger animals are on the left side of the figure and older ones on the right. The greater part of the interspecific variation was accounted for by factor 2, whereas factor 1 was mainly an age-related size component. Specimens *a* (*M. californicus* MVZ 60182) and *b* (*M. ochrogaster* UI 32595) of Figure 5 are outliers. The KU specimens lie within the Illinoian population so we included them in

TABLE 3. Statistics for repeated measurements. a) mean ($\bar{x}$), standard deviation (s), coefficient of variation (CV) and standard error ($s_{\bar{x}}$) for the 10 repeated measurements of specimen No. 84940 (M. ochrogaster). b) mean standard deviation ($\bar{s}$) for the repeated measurements based on 10 specimens. Degrees of freedom in parentheses (Seven specimens were measured 5 times, two 6 times and one 10 times).

	a				b	
	$\bar{x}$	s	CV	$s_{\bar{x}}$	$\bar{s}$	(df)
L1	28.22	0.0422	0.1494	0.0133	0.0496	(47)
L2	28.13	0.0483	0.1717	0.0153	0.0406	(47)
L3	27.74	0.0516	0.1863	0.0163	0.0534	(42)
L4	22.02	0.0632	0.2872	0.0200	0.0460	(40)
L5	17.25	0.0527	0.3055	0.0167	0.0563	(47)
L6	8.34	0.0516	0.6192	0.0163	0.0617	(46)
L7	6.81	0.0568	0.8335	0.0180	0.0519	(47)
L8	6.27	0.0483	0.7704	0.0153	0.0503	(47)
L9	5.00	0.0000	0.0000	0.0000	0.0549	(47)
L10	2.78	0.0422	1.5167	0.0133	0.0405	(47)
L11	10.00	0.0000	0.0000	0.0000	0.0462	(45)
L12	17.69	0.0316	0.1788	0.0100	0.0432	(47)
L13	7.55	0.0527	0.6981	0.0167	0.0600	(40)
L14	10.98	0.1033	0.9406	0.0327	0.0960	(47)
L15	5.15	0.1179	2.2884	0.0373	0.1221	(47)
L16	3.07	0.1252	4.0771	0.0396	0.0844	(47)
L17	10.63	0.1767	1.6623	0.0559	0.0977	(45)
L18	4.63	0.0483	1.0433	0.0153	0.1001	(43)
L19	5.97	0.0949	1.5891	0.0300	0.0558	(47)
L20	12.65	0.0707	0.5590	0.0224	0.0604	(40)
L21	3.67	0.0483	1.3162	0.0153	0.0756	(47)
L22	12.15	0.1080	0.8890	0.0342	0.0706	(47)
L23	3.38	0.0422	1.2474	0.0133	0.0495	(36)
L24	6.56	0.0516	0.7871	0.0163	0.0508	(36)
L25	15.99	0.0316	0.1979	0.0100	0.0812	(40)
L26	6.56	0.0516	0.7872	0.0163	0.0460	(40)
B1	3.34	0.0516	1.5461	0.0163	0.0331	(46)
B2	5.60	0.0000	0.0000	0.0000	0.0379	(43)
B3	16.69	0.0316	0.1895	0.0100	0.0384	(47)
B4	4.30	0.0000	0.0000	0.0000	0.0422	(47)
B5	12.77	0.0483	0.3783	0.0153	0.0526	(41)
B6	1.28	0.0422	3.2940	0.0133	0.0261	(47)
B7	2.20	0.0471	2.1427	0.0149	0.0537	(36)
B8	4.26	0.0516	1.2122	0.0163	0.0613	(43)
B9	3.09	0.0316	1.0234	0.0100	0.0835	(46)
B10	8.79	0.0568	0.6458	0.0180	0.0678	(42)
B11	5.81	0.0568	0.9770	0.0180	0.0477	(43)
B12	4.52	0.0422	0.9328	0.0133	0.0444	(43)
B13	3.12	0.0632	2.0271	0.0200	0.0494	(47)
B14	3.51	0.0316	0.9009	0.0100	0.0225	(40)
H1	10.74	0.0516	0.4808	0.0163	0.0363	(40)
H2	7.84	0.0699	0.8918	0.0221	0.0649	(40)
H3	8.76	0.0699	0.7982	0.0221	0.0723	(40)
H4	1.76	0.0516	2.9341	0.0163	0.0529	(47)
H5	4.32	0.0632	1.4640	0.0200	0.0170	(28)
H6	8.61	0.0316	0.3673	0.0100	0.0802	(36)
H7	5.88	0.0422	0.7171	0.0133	0.0547	(39)
H8	5.70	0.0471	0.8270	0.0149	0.0447	(47)

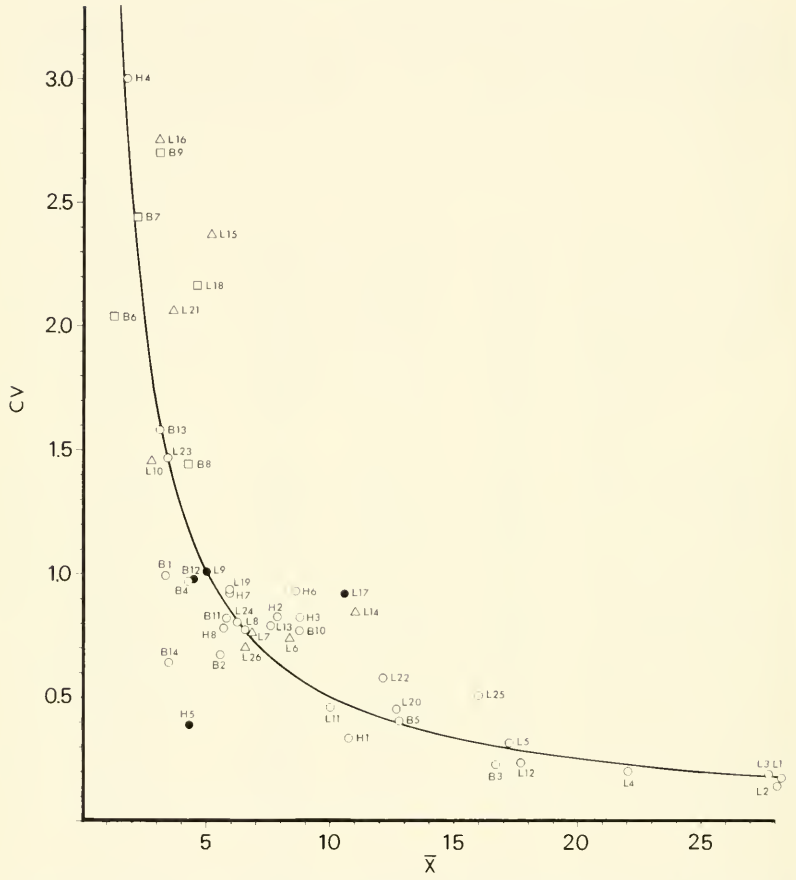

FIGURE 4.—Plot of average $\overline{CV}$ against $\bar{x}$ (mean for the 10 repeated skull measurements of specimen No. 84940), and according to different measuring techniques. Open circle—normal dial calipers (outside); solid circles—normal dial calipers (inside); triangles—needle-point calipers; squares—micrometer.

further analyses. All variables with loadings greater than 0.500 on factor 2 were also selected by the stepwise discriminant analysis program (BMDP-7M) to separate the species (see below; Table 6), with the exception of L10, L15, and L24. Loadings for the first three factors (analyses performed on each species treated separately) show that approximately 66% of total variation in *M. ochrogaster* and 68% in *M. californicus* can also be interpreted as an age related size factor. Factor 2 accounts for only 4.5% and 6.6% of the total variation in each species, respectively; its interpretation is somewhat difficult. Most variables with high loadings are those selected in the discriminant analysis to separate the sexes (see below; Table 8), with the exception of B12 in *M. ochrogaster* and L16, L24 and B14 in *M. californicus*. A PC-analysis by sex for each

TABLE 4. Loadings of variables on first five PCs from a PC-Analysis on all specimens (n = 478) of *M. californicus* and *M. ochrogaster* (males + females) using the correlation matrix. Log_{10}-transformation of all variables. ** loadings greater or equal to 0.750. * loadings greater or equal to 0.500 but less than 0.750. Other values—loadings greater or equal to 0.250 but less than 0.500. 0.—loadings less than 0.250. VP—variance proportion explained by each component. %—cumulative percentage of variance explained by each component.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
L1	0.980**	-0.	-0.	-0.	-0.
L2	0.981**	-0.	-0.	-0.	-0.
L3	0.979**	-0.	-0.	-0.	-0.
L4	0.977**	-0.	-0.	-0.	-0.
L5	0.974**	-0.	-0.	-0.	-0.
L6	0.891**	-0.365	-0.	-0.	-0.
L7	0.894**	0.284	0.	0.	0.
L8	0.941**	0.	0.	-0.	0.
L9	0.871**	0.	0.263	-0.	-0.
L10	0.658*	-0.551*	-0.329	-0.	-0.
L11	0.961**	-0.	-0.	-0.	0.
L12	0.982**	-0.	-0.	-0.	-0.
L13	0.939**	0.	-0.	-0.	-0.
L14	0.651*	-0.518*	0.	0.	-0.
L15	0.	-0.655*	0.416	-0.	0.
L16	0.318	0.836**	-0.282	0.	-0.
L17	0.908**	-0.	-0.	-0.	-0.
L18	0.938**	0.	-0.	-0.	-0.
L19	0.920**	0.	0.	-0.	0.
L20	0.785**	-0.528*	-0.	-0.	-0.
L21	0.548*	-0.347	-0.	0.285	-0.
L22	0.962**	-0.	0.	-0.	-0.
L23	0.630*	-0.318	-0.	0.377	-0.259
L24	0.613*	0.554*	-0.356	0.	-0.
L25	0.955**	-0.	0.	-0.	-0.
L26	0.893**	0.	0.	-0.	0.
B1	0.780**	0.	0.	0.273	0.
B3	0.972**	-0.	-0.	-0.	0.
B4	-0.	-0.751**	-0.	0.	0.464
B5	0.955**	-0.	-0.	0.	0.
B6	0.327	0.750**	0.307	0.	-0.
B7	0.	0.	0.578*	0.555*	-0.
B8	0.793**	-0.	0.	0.	0.
B9	0.589*	0.	0.300	0.	0.335
B10	0.866**	0.288	-0.	-0.	0.
B11	0.729*	0.491	0.	0.	0.
B12	0.403	0.648*	0.	-0.	0.
B13	0.707*	-0.582*	0.	-0.	0.
B14	0.335	0.354	-0.378	0.419	0.476
H1	0.892**	0.	-0.	0.	0.
H2	0.783**	0.286	-0.	0.	0.
H3	0.957**	-0.	-0.	-0.	-0.
H4	0.740*	0.365	-0.	-0.	0.
H5	0.	0.755**	0.	-0.292	0.
H6	0.948**	0.	0.	-0.	-0.
H7	0.883**	0.	0.	-0.	-0.
H8	0.854**	-0.	0.307	0.	0.
HB	0.912**	0.	-0.	-0.	-0.
CRANW	0.974**	-0.	-0.	-0.	0.
MANDW	0.949**	-0.	-0.	-0.	0.
VP	32.326	6.324	1.753	1.242	1.009
% (cumul.)	64.65	77.30	80.81	83.29	85.31

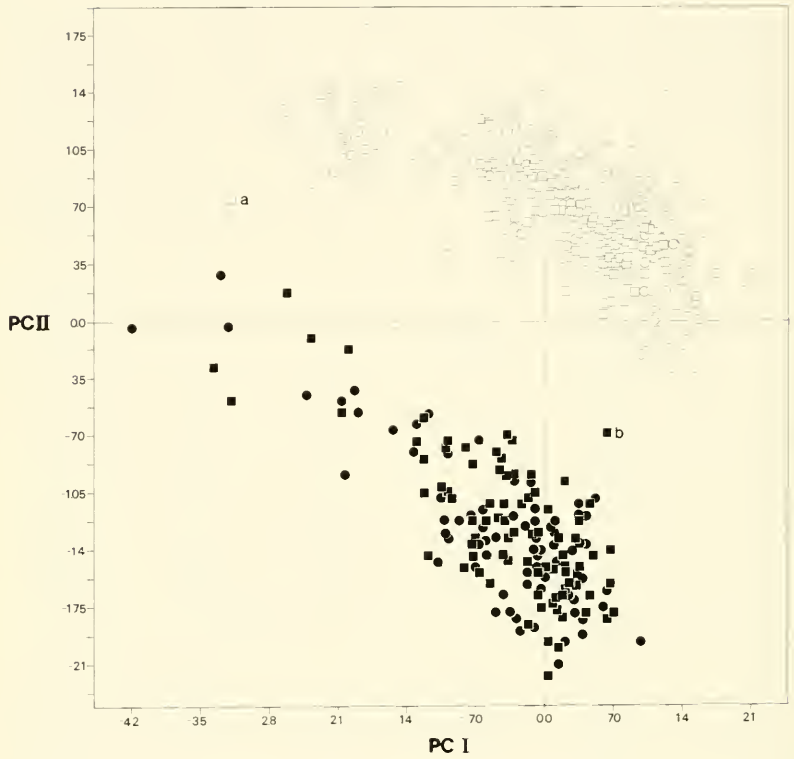

FIGURE 5.—Plot of PC I against PC II from PC-Analysis on all specimens (n = 478) based on the correlation matrix. Log_{10}-transformation of all variables. Open symbols—*M. californicus;* Solid symbols—*M. ochrogaster;* Squares—males; circles—females. For a and b, see explanations in the text.

species revealed that the most variation was concentrated in factor 1. The other components are difficult to interpret, and no clear pattern is visible from the plots.

PC-analyses using only cranial or mandibular measurements were performed on the whole sample (n = 478). In the first case, the distinction between the species is almost as good as when all variables were employed. In the second case, no clear pattern appeared when the first two factors were plotted against each other; the mandibular characters chosen do not convey much information about the variation between the two species in that part of the skull.

The correlation with age for the first five components of PC-analyses performed on different groups of specimens (Table 5) showed that when all individuals are taken together, the first two components are highly correlated with age. Approximately 50 percent ($R^2 = 0.5$) of the variation accounted for by factor 1 and 25 percent by factor 2 is age variation. When taking specimens by age-classes, about 67 percent of factor 1 and 10

percent of factor 2 is age variation in age-class 1. In age-classes 2 and 3, only about 3 and 13 percent, and 7 and 20 percent, respectively, of the variation in the first two factors is due to age. Taking age-classes 2 and 3 together, 46 percent of factor 2 is age variation and the other components only explain a very low proportion of age variation. This is somewhat peculiar; in age-class 1, the greatest source of variation is age-related size, while in classes 2 and 3 it is the interspecific differences. We have to keep in mind, however, that in all analyses used to compute the correlation

TABLE 5. Correlations with age of the first five PCs extracted for different subsamples, using the correlation matrix, except in the first case where the covariance matrix (COVA) was used. Log_{10}-transformation of all variables. ** significant to the 0.01 level. * significant to the 0.05 level.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	n
Case						
1. *Microtus ochrogaster* + *Microtus californicus* (COVA)	.745**	−.495**	.019	−.004	−.103*	478
2. *Microtus ochrogaster* + *Microtus californicus*	.720**	−.524**	.024	.095*	.022	478
3. *Microtus ochrogaster* + *Microtus californicus* Age-Class 1	.818**	−.321**	−.111	−.179*	.026	192
4. *Microtus ochrogaster* + *Microtus californicus* Age-Class 2	.180**	.363**	.011	.156*	.094	209
5. *Microtus ochrogaster* + *Microtus californicus* Age-Class 3	.263*	.445**	.095	−.205	.013	77
6. *Microtus ochrogaster* + *Microtus californicus* Age-Class 2 + 3	−.039	.679**	.027	.111	−.097	286
7. *Microtus californicus*	.875**	−.033	−.109	−.118*	−.044	314
8. *Microtus californicus* males only	.970**	−.051	−.103	−.012	−.043	173
9. *Microtus californicus* females only	.860**	.009	−.215**	−.066	−.025	141
10. *Microtus ochrogaster*	.880**	.075	−.090	−.120	−.043	164
11. *Microtus ochrogaster* males only	.884**	.050	−.137	−.068	.017	88
12. *Microtus ochrogaster* females only	.885**	.090	−.016	−.146	.048	76

coefficients of Table 5, the first factor explains about 65 percent of the total variation, whereas the second factor accounts for only about 6% when both species are taken together, and around 2 and 3 percent respectively, when *M. ochrogaster* or *M. californicus* are considered separately. That means, for instance, that in case 2 of Table 5, only 33.5 percent ($=0.720^2 \times 64.65$) of the total age variation is explained by factor 1. When the species are treated separately, we find the following results: *M. ochrogaster:* 51 percent ($=0.88^2 \times 65.8$) and *M. californicus* 52.1% ($=0.875^2 \times 68.0$). Thus, factor 1 explains about half of age variation when PC-analysis is performed on each species separately, but when the two species are combined, it is only in the order of 30-35%.

Finally, a PC-analysis using specimens of both species from age-class 2 only was performed. Animals in that age-class are from two and one-half to seven months old. Most specimens of arvicoline rodents used in taxonomic studies are of that age and are sexually mature at two to three months, though not full-grown. The correlations of factors 1 and 2 with age are rather low, so that in this case age variation accounted for by factor

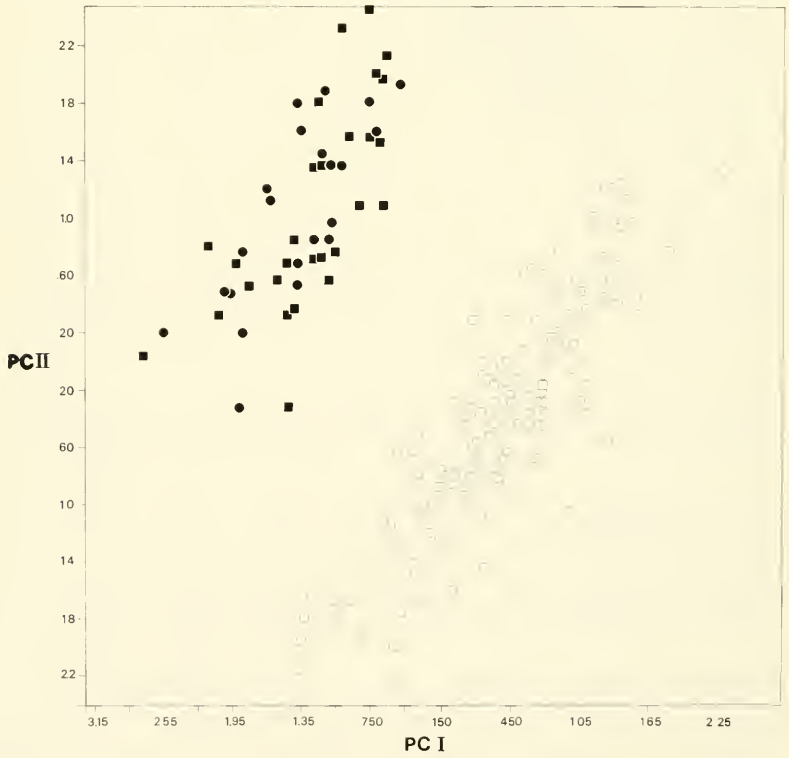

FIGURE 6.—Plot of PC 1 against PC II from PC-Analysis on specimens from age-class 2 (n = 209), based on the correlation matrix. Log_{10}-transformation of all variables (same symbols as in Figure 5).

1 and 2 is less than 3 percent. Factors 1 and 2 were plotted against each other in Figure 6. The species separate very well; two KU specimens are outliers, but still group with *M. ochrogaster.*

Discriminant function analysis (DF-analysis)

Specific differences—Each species was considered as a group; males and females were analyzed separately as well as together for the three age classes. Results of the DF-analysis for the first five steps (Table 6) reveal that different variables were chosen as best discriminators for the various age-classes in both sexes. Some of them, however, (L16, B4, B6, B11, B12, B14, and H5) were selected in several groups. H5 was a good discriminator in females of age-classes 1 and 2 only. *Microtus californicus* was larger than *M. ochrogaster* in the following measurements: interparietal length (L16), incisive foramen width (B6), condylar width (B11), foramen magnum width (B12), and anteorbital constriction width (B14).

TABLE 6. Discriminant analysis between *M. californicus* and *M. ochrogaster* according to sex and age-classes. First 5 steps. Untransformed data. Var.—variable taken at each step; F—approximation to U-statistic; %—average percentage of correct classification.

	Steps		1	2	3	4	5
	Age-Class						
		Var.	B6	L16	B4	L21	B11
	1	F	165.8	166.3	170.2	152.3	150.1
		%	89.0	96.3	97.2	98.2	100.0
		Var.	B11	B4	L16	B12	L14
	2	F	364.8	313.9	331.8	302.1	264.6
MALES		%	97.4	99.1	100.0	100.0	100.0
		Var.	L13	B4	L8	CRANW	L17
	3	F	128.9	128.0	198.6	187.7	184.7
		%	100.0	100.0	100.0	100.0	100.0
		Var.	L16	B4	B12	B6	B13
	1-3	F	559.0	618.3	600.3	530.8	463.0
		%	93.9	98.9	98.9	99.2	99.6
		Var.	L16	B6	H5	B4	B14
	1	F	133.5	131.3	110.1	91.7	90.2
		%	96.4	96.4	98.8	98.8	97.6
		Var.	B4	L7	H5	L20	B11
	2	F	185.8	193.0	253.3	221.9	209.3
FEMALES		%	95.7	100.0	100.0	100.0	100.0
		Var.	HB	H4	B11	B4	B6
	3	F	156.8	116.1	113.1	105.5	124.3
		%	100.0	100.0	100.0	100.0	100.0
		Var.	L16	B6	B4	B12	B14
	1-3	F	487.8	522.5	499.9	460.7	412.1
		%	96.3	98.6	99.1	99.5	99.1
		Var.	L16	B4	B12	B6	B14
MALES + FEMALES	1-3	F	1050.9	1098.3	1107.0	993.7	869.9
		%	96.2	99.0	99.0	99.4	99.4

Interorbital width (B4) showed the opposite trend. It is interesting that most good discriminators are width measurements. The distinction between the species improved with age for both sexes. Results of age-class 3 have to be interpreted carefully because of the small sample size in *M. californicus*. The distribution of the values for the first canonical variate (CNVR1) for females (Fig. 7) computed with 10 variables showed the species well separated. Table 7 reports the percentages of correct classification in each group for the first five steps or until a 100 percent correct classification is achieved. The jackknifed classification has been used throughout: each case is classified into a group according to the classification functions computed from all the data, except those from the case being classified. In females from age-class 1, and when all age-classes are taken together, a few specimens of *M. californicus* are misclassified as *M. ochrogaster*, whereas no *M. ochrogaster* are misclassified. This is not true for males, except in age-class 2, where the same proportion of misclassification occurred in both species.

Sexual differences—Each sex was considered as a group, and each species was analyzed separately for the different age-classes. Table 8 gives the results of the DF-analyses for the first five steps (except for *M.*

TABLE 7. Discriminant analysis between *M. californicus* and *M. ochrogaster* according to sex and age-classes: Percentages of correct classification for each group. First 5 steps or until a 100% of correct classification is reached. Untransformed data. C/C = *M. californicus* classified correctly as *M. californicus*, C/O = *M. californicus* classified as *M. ochrogaster*, etc.

	MALES				FEMALES			
	C/C	C/O	O/C	O/O	C/C	C/O	O/C	O/O
	85.00	15.00	0.00	100.00	95.16	4.84	0.00	100.00
	96.25	3.75	3.45	96.55	95.16	4.84	0.00	100.00
Age-Class 1	97.50	2.50	3.45	96.55	98.39	1.61	0.00	100.00
	98.75	1.25	3.45	96.55	98.39	1.61	0.00	100.00
	100.00	0.00	0.00	100.00	96.77	3.23	0.00	100.00
	96.51	3.49	0.00	100.00	97.22	2.78	9.09	90.91
Age-Class 2	98.84	1.16	0.00	100.00	100.00	0.00	0.00	100.00
	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00
Age-Class 3	100.00	0.00	0.00	100.00	100.00	0.00	0.00	100.00
	93.06	6.94	4.55	95.45	97.16	2.84	5.26	94.74
	99.42	0.58	2.27	97.73	97.87	2.13	0.00	100.00
Age-Class 1-3	98.84	1.16	1.14	98.86	98.58	1.42	0.00	100.00
	99.42	0.58	1.14	98.86	99.29	0.71	0.00	100.00
	100.00	0.00	1.14	98.86	98.58	1.42	0.00	100.00

	MALES + FEMALES			
	C/C	C/O	O/C	O/O
	97.45	2.55	6.10	93.90
	99.36	0.64	1.83	98.17
Age-Class 1-3	98.73	1.27	0.61	99.39
	99.36	0.64	0.61	99.39
	99.04	0.96	0.00	100.00

TABLE 8. Discriminant analysis between males and females in *M. californicus* and *M. ochrogaster*, respectively, for the different age-classes. First 10 steps. Untransformed data. A variable with a − sign means that it has been removed in the stepwise process. For further explanation see Table 6.

M. ochrogaster Steps		1	2	3	4	5
Age-Class						
	Var.	B14	B4	B7	L24	B13
1	F	15.4	9.2	7.3	6.6	5.7
	%	74.0	74.0	72.0	76.0	82.0
	Var.	B3	H1	H2	B14	H7
2	F	5.9	6.1	6.3	5.5	4.9
	%	64.7	70.6	78.4	72.5	72.5
	Var.	B4	L19	HB	B13	MANDW
3	F	10.3	8.4	8.2	8.0	7.2
	%	65.1	68.3	74.6	76.2	77.8
	Var.	B14	L14	L9	MANDW	CRANW
1-3	F	11.8	7.3	8.8	8.0	9.2
	%	59.1	61.6	64.0	65.2	70.1
M. californicus						
	Var.	B11	L8	H2	B7	B6
1	F	7.9	9.9	9.5	8.5	8.5
	%	60.6	67.6	70.4	67.6	71.1
	Var.	B5	MANDW	B10	L13	H2
2	F	77.2	53.7	42.1	35.4	31.7
	%	74.1	77.8	81.6	84.2	84.2
	Var.	L18	HB	B8		
3	F	9.6	12.8	33.3		
	%	78.6	92.9	100.0		
	Var.	H2	L8	B11	B5	MANDW
1-3	F	24.9	26.1	29.7	27.0	24.9
	%	63.1	65.6	71.3	71.0	73.6

californicus age-class 3, where a 100% of correct classification was achieved after three steps). With five variables, the percentage classified correctly was between 70 and 85 percent. Age-class three in *M. californicus* was an exception probably due to the small sample size. The sexes do not separate well, even with ten variables (Fig. 8).

Variables which best separated the sexes are not the same in each species, except for MANDW. In *M. ochrogaster*, L9, L14, B4, B13, B14 and MANDW were selected, while in *M. californicus* they were L8, B5, B11, H2 and MANDW. Sexual dimorphism is therefore expressed somewhat differently in each species. *M. ochrogaster* males show larger dimensions than females in foramen length (L9), frontal length (L14), interorbital width (B4), incisor width (B13) and anteorbital constriction (B14). Mandibular weight is greater in young males than females and lower in older males than in females. Hoffmeister and Getz (1968) did not report significant sexual dimorphism in measurements. Table 9 gives the

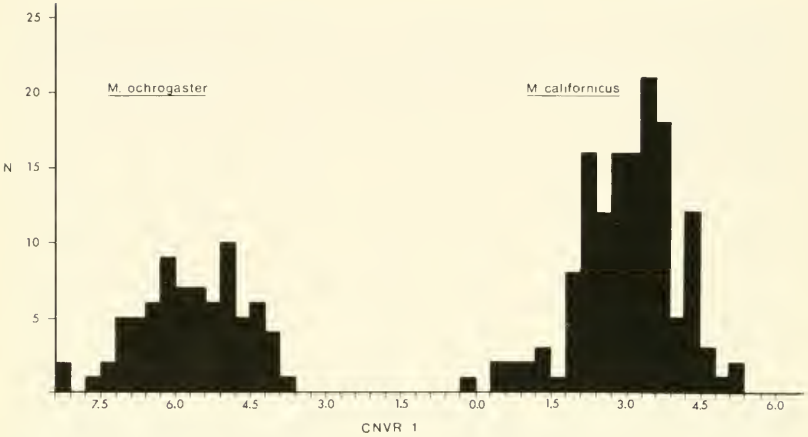

FIGURE 7.—Distribution of values for the first canonical variate (CNVR1) in DF-Analysis between *M. californicus* and *M. ochrogaster* females. Untransformed data, 10 variables used.

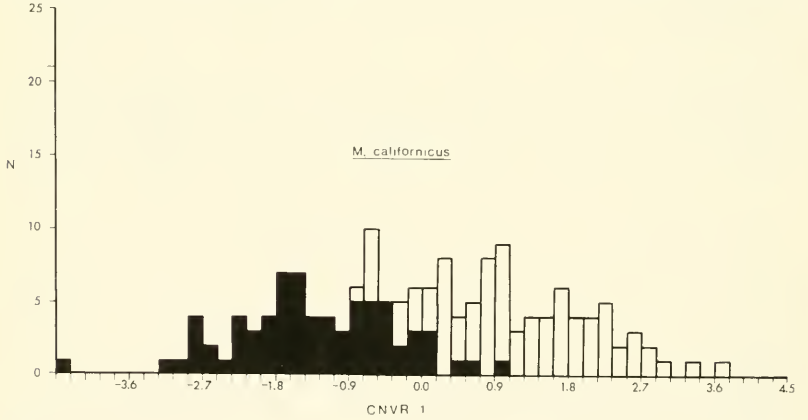

FIGURE 8.—Distribution of values for the first canonical variate (CNVR1) in DF-Analysis between males (open) and females (solid) of *M. californicus* from age-class 2. Untransformed data, 10 variables used.

percentages of correct classification (jackknifed) for the first ten steps, or until a 100 percent correct classification was achieved.

Interspecific differences by sex—Four groups were considered in this analysis: males and females of each species. Table 10a gives the results for that analysis. The best discriminators were the same variables selected in the discriminant functions between species (see above) except that H5 was not entered and HB was more important. This result is not surprising because the greater part of the variation was due to interspecific differences. Thus, variables that are good species discriminators also are more important in this analysis. The classification also improves with age. Figure 9 represents the first two canonical variates (CNVR1 and CNVR2)

plotted against each other. The species are distinct, whereas the sexes showed considerable overlap. *M. californicus* specimens *a* (MVZ 60182) and *b* (MVZ 142) are outliers. The former is the same as specimen *a* in Figure 5. Table 10b gives the results of a comparison including animals of both species, but belonging to different age-classes. *M. californicus* is

TABLE 9. Discriminant analysis between males and females in *M. californicus* and *M. ochrogaster*, respectively for the different age-classes: Percentages of correct classification for each group. First 10 steps or until a 100% correct classification is reached. Untransformed data. M/M = males classified correctly as males, M/F = males classified as females, etc.

	Microtus ochrogasater				*Microtus californicus*			
	M/M	M/F	F/M	F/F	M/M	M/F	F/M	F/F
Age-Class 1	68.97	31.03	19.05	80.95	66.25	33.75	46.77	53.23
	72.41	27.59	23.81	76.19	67.50	32.50	32.26	67.74
	68.97	31.03	23.81	76.19	70.00	30.00	29.03	70.97
	75.86	24.14	23.81	76.19	67.50	32.50	32.26	67.74
	82.76	17.24	19.05	80.95	71.25	28.75	29.03	70.97
	79.31	20.69	9.52	90.48	68.75	31.25	29.03	70.97
	86.21	13.79	14.29	85.71	72.50	27.50	25.81	74.19
	79.31	20.69	14.29	85.71	72.50	27.50	17.74	82.26
	79.31	20.69	14.29	85.71	75.00	25.00	19.35	80.65
	86.21	13.79	9.52	90.48				
Age-Class 2	58.62	41.38	37.27	72.73	73.26	26.74	25.00	75.00
	65.52	34.48	22.73	77.27	75.58	24.42	19.44	80.56
	75.86	24.14	18.18	81.82	81.40	18.60	18.06	81.94
	68.97	31.03	22.73	77.27	83.72	16.28	15.28	84.72
	72.41	27.59	27.27	72.73	83.72	16.28	15.28	84.72
	75.86	24.14	13.64	86.36	84.88	15.12	15.28	84.72
	82.76	17.24	22.73	77.27	89.53	10.47	15.28	84.72
	82.76	17.24	18.18	81.82	90.70	9.30	8.33	91.67
	79.31	20.69	13.64	86.36	88.37	11.63	12.50	87.50
	79.31	20.69	9.09	90.91	87.21	12.79	11.11	88.89
Age-Class 3	56.67	43.33	27.27	72.73	85.71	14.29	28.57	71.43
	70.00	30.00	33.33	66.67	100.00	0.00	14.29	85.71
	73.33	26.67	24.24	75.76	100.00	0.00	0.00	100.00
	73.33	26.67	21.21	78.79	100.00	0.00	0.00	100.00
	73.33	26.67	18.18	81.82				
	80.00	20.00	15.15	84.85				
	80.00	20.00	15.15	84.85				
	83.33	16.67	18.18	81.82				
	83.33	16.67	18.18	81.82				
	86.67	13.33	15.15	84.85				
Age-Class 1-3	67.05	32.95	50.00	50.00	67.05	32.95	41.84	58.16
	60.23	39.77	36.84	63.16	61.27	38.73	29.08	70.92
	61.36	38.64	32.89	67.11	71.10	28.90	28.37	71.63
	63.64	36.36	32.89	67.11	71.68	28.32	29.79	70.21
	68.18	32.82	27.63	72.37	73.99	26.01	26.95	73.05
	70.45	29.55	27.63	72.37	76.88	23.12	26.24	73.76
	73.86	26.14	23.68	76.32	76.30	23.70	23.40	76.60
	72.73	27.27	27.37	77.63	76.88	23.12	21.28	78.72
	77.27	22.73	21.05	78.95	78.61	21.39	24.11	75.89
	77.27	22.73	23.68	76.32	78.03	21.97	20.57	79.43

TABLE 10. a) Discriminant analysis between *M. californicus* and *M. ochrogaster*, and the sexes, for the different age-classes. First 10 steps. Untransformed data. A * means that at the given step a 100% correct classification for the species has been reached. For further explanation see Tables 6 and 8. b) Same as in a), but for different age combinations.

a Steps		1	2	3	4	5	6	7	8	9	10
Age-Class											
	Var.	L16	B4	B6	B14	L21	B11	B13	L7	L8*	L20
1	F	96.6	67.8	52.3	42.1	36.1	33.7	32.6	33.0	30.7	29.1
	%	49.0	62.0	62.0	61.5	60.9	64.1	66.1	66.1	66.1	65.6
	Var.	B11	B4	L16*	B12	HB	L19	B5	L14	-HB	B6
2	F	179.9	106.7	83.8	72.7	62.6	55.1	50.7	46.6	52.3	48.1
	%	61.2	65.6	68.4	70.8	72.7	74.6	79.4	78.5	76.6	77.5
	Var.	HB	B4*	B6	CRANW	MANDW	B10	B12	-B6	L16	L14
3	F	85.7	49.2	36.2	29.7	26.2	23.7	21.8	24.2	22.4	20.8
	%	61.0	67.5	66.2	72.7	80.5	80.5	79.2	77.9	77.9	79.2
	Var.	L16	B4	B12	B6	B14	L14	L7*	L20	B11	L8
1-3	F	349.3	220.6	175.4	140.7	118.2	103.0	95.6	90.2	85.1	78.9
	%	50.0	57.1	56.5	56.7	57.5	60.3	59.0	61.3	67.6	69.2
b											
M. californicus age-class 2 and *M. ochrogaster* age-class 1	Var.	HB	B6	B4*	B12	L16	L19	L14	B5	B13	H7
	V	250.7	118.7	86.5	72.6	66.4	60.2	56.1	52.6	48.5	45.2
	%	71.3	70.8	72.7	72.7	72.7	76.6	75.1	78.9	76.6	77.5
M. californicus age-class 1 and *M. ochrogaster* age-class 2	Var.	B4	L16	B13	B11*	B6	B14	L8	L20	CRANW	-L16
	F	97.3	73.7	61.8	62.9	54.4	48.3	43.1	40.9	38.4	42.6
	%	46.9	56.2	58.8	64.9	63.9	64.9	70.6	72.2	70.1	69.6

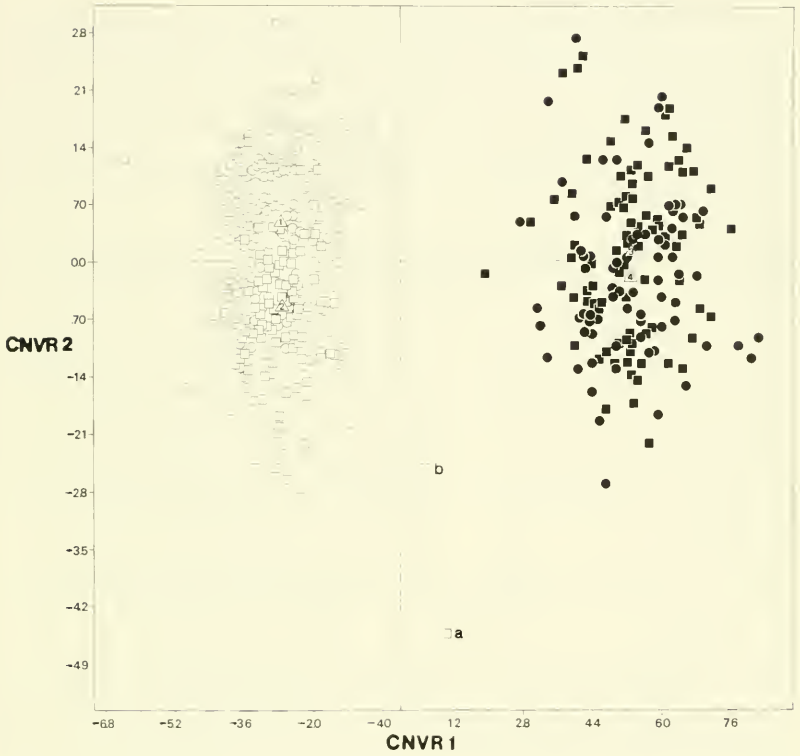

FIGURE 9.—Distribution of values for canonical variates 1 (CNVR1) and 2 (CNVR2) in DF-Analysis between *M. californicus* and *M. ochrogaster*, and the sexes. Untransformed data, 10 variables used. Numbered triangles are group centroids (1, 3 male; 2, 4 female). For a and b, see explanations in the text. Same symbols as in Figure 5.

larger than *M. ochrogaster* in most measurements, so by combining *M. californicus* of age-class 2 and *M. ochrogaster* of age-class 1, the size difference is exaggerated, while by combining *M. californicus* of age-class 1 and *M. ochrogaster* of age-class 2, the size difference is minimized. We know this because in the first case only three variables are needed, among them HB, a good size indicator, to classify correctly all specimens according to species, whereas in the second case four are needed. These variables (B4, L16, B13 and B11) are less age-dependent. Table 11 gives the percentages of correct classification (jackknifed) with ten variables for the different age classes, except in part B, where only nine variables were used because the F-to enter was less than 1.0 after step 9. In general, the correct classification of specimens according to sex is not as good as when sex alone is considered (see above). This result was expected because sexual and species variation are of different orders of magnitude, so that the former is overshadowed by the latter.

Canonical Correlation Analysis

The following comparisons were made for cranial measurements only:

TABLE 11. Discriminant analysis between *M. californicus* and *M. ochrogaster*, and the sexes, for the different age-classes: Percentages of correct classification. First 10 steps. Untransformed data. CM/CM = *M. californicus* male classified as such, CM/OM = *M. californicus* male classified as *M. ochrogaster* male, etc.

	CM/CM	CM/CF	CM/OM	CM/OF	CF/CM	CF/CF	CF/OM	CF/OF	OM/CM	OM/CF	OM/OM	OM/OF	OF/CM	OF/CF	OF/OM	OF/OF
A																
	45.00	46.25	8.75	0.00	45.15	50.00	4.84	0.00	10.34	0.00	51.72	37.93	0.00	0.00	42.86	57.14
	56.25	42.50	0.00	1.25	35.48	59.68	0.00	4.84	3.45	0.00	72.41	24.14	0.00	0.00	23.81	76.19
	56.25	42.50	1.25	0.00	35.48	59.68	3.23	1.61	3.45	0.00	72.41	24.14	0.00	0.00	23.81	76.19
	57.50	41.25	1.25	0.00	35.48	59.68	3.23	1.61	3.45	0.00	62.07	34.48	0.00	0.00	19.05	80.95
	55.00	43.75	1.25	0.00	40.32	56.45	3.23	0.00	3.45	0.00	72.41	24.14	0.00	0.00	19.05	80.95
Age-Class 1	61.25	38.75	0.00	0.00	38.72	59.68	0.00	1.61	0.00	0.00	68.97	31.03	0.00	0.00	19.05	80.95
	65.00	35.00	0.00	0.00	33.87	64.52	0.00	1.61	0.00	0.00	68.97	31.03	0.00	0.00	28.57	71.43
	66.25	33.75	0.00	0.00	33.87	64.52	0.00	1.61	0.00	0.00	68.97	31.03	0.00	0.00	33.33	66.67
	62.50	37.50	0.00	0.00	33.87	66.13	0.00	0.00	0.00	0.00	75.86	24.14	0.00	0.00	33.33	66.67
	62.50	37.50	0.00	0.00	33.87	66.13	0.00	0.00	0.00	0.00	68.97	31.03	0.00	0.00	28.57	71.43
	69.77	29.07	0.00	1.16	26.39	65.28	0.00	8.33	0.00	3.45	41.38	55.17	0.00	4.55	54.55	40.91
	73.26	25.58	1.16	0.00	30.56	68.06	1.39	0.00	0.00	0.00	37.93	62.07	0.00	0.00	36.36	63.64
	77.91	22.09	0.00	0.00	33.33	66.67	0.00	0.00	0.00	0.00	51.72	48.28	0.00	0.00	40.91	59.09
	74.42	25.58	0.00	0.00	30.56	69.44	0.00	0.00	0.00	0.00	68.97	31.03	0.00	0.00	36.36	63.64
	75.58	24.42	0.00	0.00	23.61	76.39	0.00	0.00	0.00	0.00	68.97	31.03	0.00	0.00	45.45	54.55
Age-Class 2	75.58	24.42	0.00	0.00	19.44	80.56	0.00	0.00	0.00	0.00	68.97	31.03	0.00	0.00	40.91	59.09
	81.40	18.60	0.00	0.00	16.67	83.33	0.00	0.00	0.00	0.00	72.41	27.59	0.00	0.00	31.82	68.18
	81.40	18.60	0.00	0.00	16.67	83.33	0.00	0.00	0.00	0.00	72.41	27.59	0.00	0.00	40.91	59.09
	77.91	22.09	0.00	0.00	19.44	80.56	0.00	0.00	0.00	0.00	79.31	20.69	0.00	0.00	40.91	59.09
	82.56	17.44	0.00	0.00	18.06	81.94	0.00	0.00	0.00	0.00	75.86	24.14	0.00	0.00	40.91	59.09
	71.43	28.57	0.00	0.00	42.86	57.14	0.00	0.00	3.33	0.00	53.34	43.33	0.00	0.00	33.33	66.67
	57.14	42.86	0.00	0.00	57.14	42.86	0.00	0.00	0.00	0.00	63.33	36.67	0.00	0.00	21.21	78.79
	42.86	57.14	0.00	0.00	57.14	42.86	0.00	0.00	0.00	0.00	63.33	36.67	0.00	0.00	21.21	78.79
	85.71	14.29	0.00	0.00	57.14	42.86	0.00	0.00	0.00	0.00	76.67	23.33	0.00	0.00	27.27	72.73
	85.71	14.29	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	76.67	23.33	0.00	0.00	21.21	78.79
Age-Class 3	85.71	14.29	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	76.67	23.33	0.00	0.00	21.21	78.79
	100.00	0.00	0.00	0.00	14.29	85.71	0.00	0.00	0.00	0.00	76.67	23.33	0.00	0.00	24.24	75.76
	100.00	0.00	0.00	0.00	14.29	85.71	0.00	0.00	0.00	0.00	73.33	26.67	0.00	0.00	21.21	78.79
	100.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	73.33	26.67	0.00	0.00	21.21	78.79
	100.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	73.33	26.67	0.00	0.00	21.21	78.79

TABLE 11. (Continued)

	51.45	46.24	2.31	0.00	49.65	47.52	2.84	0.00	0.00	6.82	47.73	45.45	0.00	5.26	40.79	53.95
	57.23	42.20	0.00	0.58	44.68	54.61	0.00	0.71	2.27	0.00	64.77	32.95	1.32	0.00	46.05	52.63
	56.65	42.20	0.00	1.16	43.97	54.61	0.71	0.71	1.14	0.00	60.23	38.64	0.00	0.00	44.74	55.26
	53.18	46.24	0.58	0.00	41.13	58.16	0.71	0.00	1.14	0.00	61.36	37.50	0.00	0.00	43.42	56.58
	52.60	46.82	0.58	0.00	39.72	58.87	0.71	0.71	0.00	0.00	61.36	38.64	0.00	0.00	38.16	61.84
Age-Class 1-3	55.49	44.51	0.00	0.00	39.01	60.99	0.00	0.00	0.00	1.14	61.36	37.50	0.00	0.00	31.58	68.42
	56.07	43.93	0.00	0.00	39.72	60.28	0.00	0.00	0.00	0.00	60.23	39.77	0.00	0.00	38.16	61.84
	55.49	44.51	0.00	0.00	38.30	61.70	0.00	0.00	0.00	0.00	67.05	32.95	0.00	0.00	32.89	67.11
	68.21	31.79	0.00	0.00	32.62	67.38	0.00	0.00	0.00	0.00	67.05	32.95	0.00	0.00	32.89	67.11
	70.52	29.48	0.00	0.00	28.37	71.63	0.00	0.00	0.00	0.00	65.91	34.09	0.00	0.00	34.21	65.79

	CM/CM	CM/CF	CM/OM	CM/OF	CF/CM	CF/CF	CF/OM	CF/OF
B								
	75.58	23.26	1.16	0.00	27.40	71.23	1.37	0.00
	70.93	29.07	0.00	0.00	26.03	72.60	1.37	0.00
Microtus californicus age-class 2 and *Microtus ochrogaster* age-class 1	70.93	29.07	0.00	0.00	26.03	73.97	0.00	0.00
	72.09	27.91	0.00	0.00	27.40	72.60	0.00	0.00
	74.42	25.58	0.00	0.00	30.14	69.86	0.00	0.00
	76.74	23.26	0.00	0.00	21.92	78.08	0.00	0.00
	76.74	23.26	0.00	0.00	23.29	76.71	0.00	0.00
	80.23	19.77	0.00	0.00	19.18	80.82	0.00	0.00
	80.23	19.77	0.00	0.00	21.92	78.08	0.00	0.00
	81.40	18.60	0.00	0.00	20.55	79.45	0.00	0.00
	45.00	46.25	8.75	0.00	35.48	54.84	8.06	1.61
	55.00	43.75	1.25	0.00	38.71	59.68	1.61	0.00
Microtus californicus age-class 1 and *Microtus ochrogaster* age-class 2	57.50	41.25	0.00	1.00	38.71	59.68	0.00	1.61
	65.00	35.00	0.00	0.00	33.87	66.13	0.00	0.00
	67.50	32.50	0.00	0.00	37.10	62.90	0.00	0.00
	66.25	33.75	0.00	0.00	37.10	62.90	0.00	0.00
	68.75	31.25	0.00	0.00	27.42	72.58	0.00	0.00
	68.75	31.25	0.00	0.00	25.81	74.19	0.00	0.00
	66.25	33.75	0.00	0.00	29.03	70.97	0.00	0.00

width vs. length, height vs. width, height vs. length, and cranial vs. mandibular measurements. The first few canonical variates of both sets show significant correlations with each other, indicating that they convey similar information about the skull. Untransformed and transformed ($\log_{10}$) data were used in a preliminary test, but the differences in the results were only minor, and untransformed data were thereafter employed.

Similar results to those obtained when taking only one species were observed. One interesting comparison, however, was that involving length and width measurements. The first canonical variate of the first set (CNVRF1) is highly correlated ($r = 0.979$) with the first one of the second set (CNVRS1). Table 12 gives the loadings on the first five canonical

TABLE 12. Canonical correlation analysis for *M. californicus* and *M. ochrogaster* (Males + females). Comparison of width (B) and length (L) measurements. First 5 canonical variates. Untransformed data. For further explanation see Table 4.

	CNVRF 1	CNVRF 2	CNVRF 3	CNVRF 4	CNVRF 5
B1	0.762**	-0.	-0.	-0.367	0.
B3	0.983**	0.	-0.	0.	0.
B4	-0.	0.801**	0.	-0.	0.
B5	0.960**	0.	-0.	-0.	0.
B6	0.284	-0.761**	-0.437	0.	-0
B7	0.	-0.	-0.544*	-0.500*	0.
B8	0.777**	0.	-0.	-0.	0.
B9	0.546*	-0.	-0.353	-0.	0.
B10	0.869**	-0.337	0.	0.	-0.262
B11	0.701*	-0.449	-0.	0.	-0.
B12	0.354	-0.609*	-0.271	0.348	0.
B13	0.717*	0.625*	-0.	0.	-0.
B14	0.304	-0.415	0.438	-0.281	0.
	CNVRS 1	CNVRS 2	CNVRS 3	CNVRS 4	CNVRS 5
L1	0.990**	0.	0.	0.	-0.
L2	0.990**	0.	-0.	0.	-0.
L3	0.978**	-0.	-0.	-0.	-0.
L4	0.985**	0.	0.	-0.	-0.
L5	0.979**	0.	-0.	0.	0.
L6	0.901**	0.334	-0.	-0.	-0.
L7	0.861**	-0.348	-0.	-0.	0.
L8	0.927**	-0.	-0.	0.	0.
L9	0.840**	-0.	-0.458	0.	-0.
L10	0.668*	0.532*	0.376	-0.	0.
L11	0.959**	-0.	-0.	-0.	0.
L12	0.986**	-0.	0.	0.	0.
L13	0.936**	-0.	-0.	-0.	-0.
L14	0.647*	0.580*	-0.	-0.	0.
L15	0.	0.659*	-0.275	-0.	0.
L16	0.317	-0.859**	0.	-0.	0.
L17	0.921**	0.	0.	0.	0.
L18	0.950**	-0.	0.	0.	-0.
L23	0.620*	0.250	-0.	-0.307	-0.
L24	0.595*	-0.604*	0.	-0.	0.

variates for each variable in each set. Animals with large dimensions in the following variables: L1, L2, L3, L4, L5, L6, L7, L8, L9, L11, L12, L13, L17, L18 (and to some extent L10, L14, L23 and L24) also have large dimensions in B1, B3, B5, B8, B10 (and to some extent B9, B11 and B13). In Figure 10, the second canonical variates (CNVRF2 and CNVRS2) are plotted against each other; two groups corresponding to the species are clearly apparent. The relationship between CNVRF2 and CNVRS2, which show a correlation of 0.888, can be summarized as follows, according to the loadings of Table 12: *M. ochrogaster* possesses relatively larger dimensions in B4, B13, L10, L14, L15 and smaller ones in B6, B11, B12, L16, L24 than does *M. californicus*. The specimens indicated by an arrow in Figure 10 are young individuals (less than 1 month old) of *M. ochrogaster*. They lie closer to the *M. californicus* than to the *M. ochrogaster* group. However, not all young specimens of *M. ochrogaster* are to be found within the *M. californicus* population, indicating that age is not the only factor determining their position.

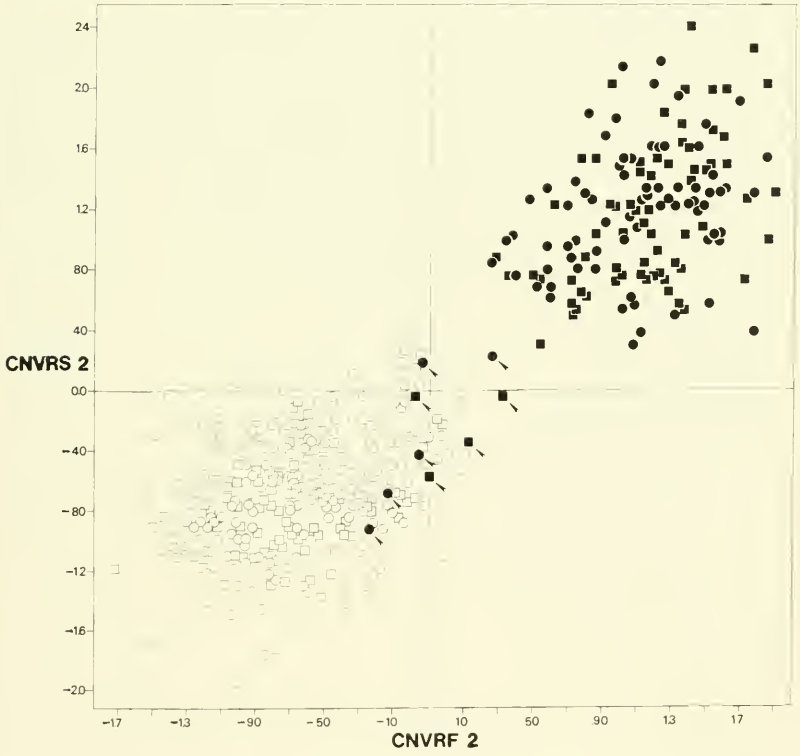

FIGURE 10.—Canonical correlation analysis on *M. californicus* and *M. ochrogaster*. Plot of canonical variate 2 of first set (CNVRF2) against canonical variate 2 of the second set (CNVRS2). Untransformed data. For individuals designated by arrow, see explanations in the text. Same symbols as in Figure 5.

Multiple regression analysis; predicting individual age

The age criteria provided by Hoffmeister and Getz (1968) for *M. ochrogaster* do not extend beyond an age of 6 weeks; older animals could not be distinguished unless eye lens weights were used. Most age criteria proposed were qualitative, such as sutures, and subject to considerable error. Lidicker and MacLean (1969) presented two complex procedures for estimating age in *M. californicus*, based on growth curves and regression analysis respectively. Their data were divided into two subsamples: animals less than and more than 100 days old. A regression analysis was then performed on each subsample, because they felt that a formula derived from the whole data set would have given poor estimators of age. Thus, to estimate the age of an animal one has to go through a series of steps leading to the formula to be used.

Our objective was to seek a simpler, more general model to predict age. First we transformed our variables (including age) into logarithms ($\log_{10}$) to linearize our data (Chatterjee and Price, 1977). With only a few exceptions, all variables showed stronger correlations with age than when they were untransformed.

Stepwise regression was first computed for our different subsamples grouped according to species and sexes (Table 13, row A). The RSQ values (multiple correlation coefficients) indicate what proportion of the variation is explained by the regression model. About 85 percent was accounted for with three variables, 88-92 percent with 10 variables and 91-96 percent with 20 variables (Table 13, row A). These values are somewhat reduced when species are combined. The increase in RSQ can be used to judge whether the inclusion of a new variable adds much to the predictive power of the regression equation and makes it possible to select the number of variables to be employed. Selection of variables in the stepwise procedure can be influenced by variables already in the equation; *i.e.* we do not know what the outcome would have been had another variable been taken first. This was pointed out by Lidicker and MacLean (1969), Daniel and Wood (1971), and Chatterjee and Price (1977). The all possible subsets regression, which computes the best subset of variables, was also available; "best" is defined as the subset with the smallest C_P. This statistic compares the residual sum of squares for the equation with all variables to that of smaller subset; the number of specimens and variables in the equation are also taken into account (Daniel and Wood, 1971; Chatterjee and Price, 1977). Results from the best subsets regression are presented in Table 13, row B. Variable names are only given as long as they correspond to those selected by stepwise regression program. Usually, the first five to six variables chosen by both programs are the same, after which some divergences occur. Some variables had to be excluded from the best subsets regression analyses because they produced a singular matrix.

Figures 11 and 12 compare the RSQ values of stepwise and best subsets regression programs for *M. californicus* and *M. ochrogaster*, respectively. If the results were identical we should observe a straight line;

TABLE 13. Multiple regression analysis. Comparison of results from programs BMDP-2R (A) and BMDP-9R (B). First 20 steps. Log_{10}-transformation of all variables (including age). For each step, RSQ = squared multiple correlation coefficient; Var. = variable entered (in 9R given only as long as it corresponds to the set selected by 2R). A – sign indicates that the variable was deleted in the stepwise process. Underlined RSQ-values– Best subset according to the C_p-criterion (In *M. californicus* males + females, best subset with 21 variables) N = number of variables not included in the analysis (9R) because of matrix singularity.

	Step:		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	N
Microtus ochrogaster males	A	Var.	B3	H6	H4	L16	B11	HB	L9	H2	H7	L23	L24	B13	L13	L7	L18	MANDW	H3	L15	H1	–L9	
		RSQ	.803	.841	.858	.870	.876	.884	.890	.895	.900	.904	.909	.912	.915	.918	.921	.925	.928	.930	.933	.932	
	B	Var.	B3	H6	H4	L16	B11	HB															
		RSQ	.803	.841	.858	.870	.876	.884	.892	.899	.902	.907	.911	.915	.919	.923	.925	.929	.931	.935	.938	.939	6
Microtus ochrogaster females	A	Var.	B3	L16	L13	L7	L23	L3	B8	B12	B10	–L13	L14	B6	L6	H2	L18	L10	B13	B4	H5	–B8	
		RSQ	.842	.864	.877	.887	.895	.904	.913	.917	.922	.921	.928	.934	.938	.941	.945	.948	.950	.952	.954	.954	
	B	Var.	B3	L16	L13	L7	L23	L17															
		RSQ	.842	.864	.877	.887	.895	.902	.909	.919	.926	.932	.937	.940	.943	.947	.949	.950	.951	.953	.954	.955	10
Microtus ochrogaster males + females	A	Var.	B3	L16	H2	L13	L23	B6	L9	H6	B11	L3	L7	HB	H4	L18	B8	H1	MANDW	L6	L15	H7	
		RSQ	.818	.846	.853	.859	.869	.877	.881	.887	.890	.895	.899	.902	.905	.908	.910	.913	.915	.918	.919	.920	
	B	Var.	B3	L16	H2	L13	L23	B6															
		RSQ	.818	.846	.853	.859	.869	.877	.882	.887	.893	.897	.900	.903	.906	.908	.912	.914	.916	.918	.919	.920	5
Microtus californicus males	A	Var.	B3	B13	H5	L1	B11	L16	L7	H4	H3	HB	L3	L4	L15	B12	–L16	L22	L26	L19	B7	L20	
		RSQ	.823	.844	.853	.860	.865	.870	.874	.878	.880	.883	.886	.888	.890	.893	.893	.895	.896	.899	901	.902	
	B	Var.	L1	B13	H5	B11	B3																
		RSQ	.817	.845	.856	.860	.865	.869	.873	.876	.878	.880	.883	.885	.888	.890	.891	.893	.894	.895	.896	.896	13
Microtus californicus females	A	Var.	MANDW	B14	H3	B11	H4	B13	B9	CRANW	H8	B10	L1	L3	L13	B5	B8	–B11	H1	L9	L12	B1	
		RSQ	.794	.818	.834	.843	.854	.860	.867	.872	.878	.883	.888	.890	.894	.897	.901	900	.904	.905	.907	.908	
	B	Var.	MANDW	B14	H3	B11	H4	B13	B9														
		RSQ	.794	.818	.834	.848	.854	.860	.867	.872	.878	.884	.886	.889	.893	.894	.897	.900	.902	.904	.906	.907	7
Microtus californicus males + females	A	Var.	MANDW	B13	B11	L22	B9	H3	H1	B3	H4	H5	B14	H7	CRANW	L19	B6	L1	L3	B1	L7	B10	
		RSQ	.789	.811	.823	.831	.838	.843	.851	.855	.859	.862	.865	.867	.870	.872	.873	.875	.877	.878	.879	.880	
	B	Var.	MANDW	B13	B11	L22	B9																
		RSQ	.789	.811	.823	.831	.838	.848	.852	.857	.861	.863	.865	.867	.868	.869	.870	.872	.873	874	.875	876	7

the results are very similar for both species. However, there are a few differences worth pointing out. In *M. californicus* males, the first variable chosen (L1) by best subsets regression is not as good as that chosen (B3) by stepwise regression; the first variable selected by stepwise regression (B3) was not included in the best subsets regression analysis because it showed a high correlation with L1, which in turn was not as highly correlated with age as B3. Thus excluding L1 instead of B3 would have been a better strategy. However, after a few steps the results became very similar again. When 10 or more variables were in the equation, stepwise regression performed slightly better than best subsets regression, due probably to excluded variables. In *M. ochrogaster* males, and to some extent in males + females, best subsets regresssion performed better than stepwise regression from the seventh variable onwards, but the differences in RSQ are less than 0.005 (=0.5%).

In regression analysis, two main approaches are possible. The first consists of finding an equation describing a relationship between a dependent variable and one or more independent ones. The fewer

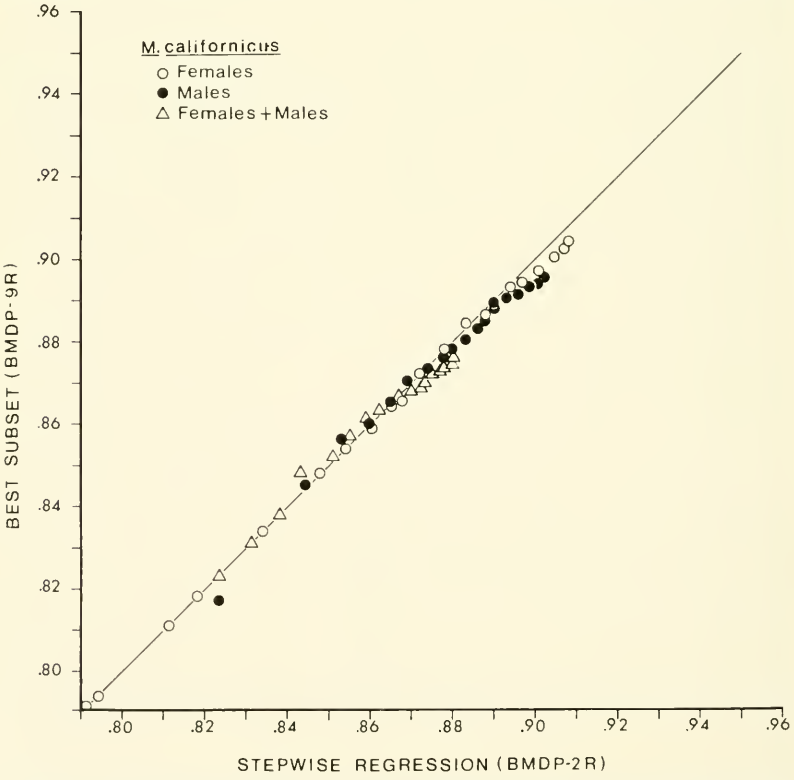

FIGURE 11.—Comparison between Best subset (program BMDP-9R) and stepwise regression (program BMDP-2R). Plot of RSQ values from both programs against each other for *M. californicus*.

variables needed for a good fit (reflected in RSQ or RMS) the more easily the relationship can be explained. The second approach concerns the predictive power of a model. It is important to minimize RMS with, if possible, a minimum number of variables; this is the approach we have used. By comparing RSQ and RMS values (Table 14), we can see that the lowest RSQ are observed for the untransformed data, and the highest ones are those in which all 50 variables have been used. RMS is usually lowest in equations with fewer variables. When the number of variables gets close to the number of specimens, as is the case for *M. ochrogaster* especially, RSQ tends toward 1 and is misleading. The adjusted RSQ (ADJRSQ) (Chatterjee and Price, 1977) which depends on the number of variables in the equation and is always lower than RSQ, gives a better idea of the goodness of fit of a model. With the exception of *M. ochrogaster* females, ADJRSQ is highest and RMS lowest for analyses using program BMDP-9R with untransformed independent variables and $\log_{10}$-age (second row of each group in Table 14, except for *M. californicus* males,

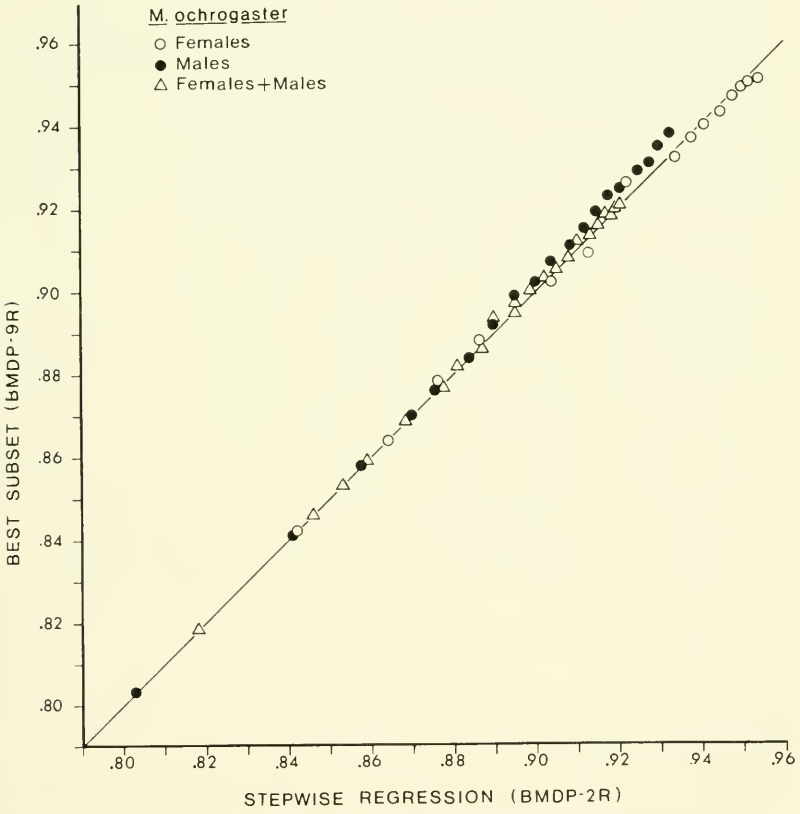

FIGURE 12.—Comparison between Best subset (program BMDP-9R) and stepwise regression (program BMDP-2R). Plot of RSQ values from both programs against each other for *M. ochrogaster*.

TABLE 14. Multiple regression analysis. Comparison of results from programs BMDP-2R and 9R. n = number of specimens; A = number of variables in analysis; B = selection type of variables; – = all variables included; a—first 10 variables selected by 2R; b—9R (without those making the matrix singular, 0.01 level); c = 9R: first 20 from best subset; C = number of variables in regression; D = method of regression; 1—simple multiple regression; 2—best subset; E = transformation; 1—raw data; 2—$\log_{10}$-age; 3—$\log_{10}$ all variables; C = Mallow's C_p; RSQ = squared correlation coefficient; ADJRSQ = adjusted RSQ; RMS = residual mean square; CD = Cook's distance (maximum); SR = number of studentized residuals greater or equal to 2.0; MD = Mahalanobis distance (maximum); F_{stat} = F-statistic with corresponding degrees of freedom (df).

	n	A	B	C	D	E	C_p	RSQ	ADJRSQ	RMS	CD	SR	MD	F_{stat}	df
M. californicus/M	173	50	–	50	1	3	–	.91399	.87874	.013533	.11	–	–	25.93	50/122
"	173	37	b	11	2	1	-0.17	.71620	.69681	1248.05	.15	8	37.79	36.94	11/161
"	173	37	b	13	2	2	3.20	.89239	.88359	.012992	.08	10	39.31	101.43	13/159
"	173	37	b	15	2	3	5.70	.89135	.88097	.013285	.11	11	51.13	85.87	15/157
"	173	10	a	10	1	3	–	.88249	.87524	.013925	.10	11	25.35	121.66	10/162
M. californicus/F	141	50	–	50	1	3	–	.92621	.88522	.011214	.12	–	–	22.59	50/90
"	141	20	c	19	2	2	19.09	.90677	.89213	.010539	.09	7	36.89	61.94	19/121
"	141	43	b	19	2	3	4.66	.90586	.89108	.010641	.09	6	36.99	61.28	19/121
"	141	10	a	10	1	3	–	.88320	.87421	.012289	.11	9	29.04	98.30	10/130
M. californicus/M + F	314	50	–	50	1	3	–	.89032	.86947	.013725	.12	–	–	42.70	50/263
"	314	20	c	18	2	2	18.22	.88019	.87288	.013367	.05	15	52.63	120.40	18/295
"	314	43	b	21	2	3	13.88	.87723	.86840	.013838	.05	17	67.95	99.35	21/292
"	314	10	a	10	1	3	–	.86238	.85784	.014948	.04	18	50.85	189.87	10/303
M. ochrogaster/M	88	50	–	50	1	3	–	.94987	.88213	.017600	.30	–	–	14.02	50/37
"	88	20	c	19	2	2	20.23	.93994	.92316	.011474	.16	2	48.34	56.01	19/68
"	88	44	b	19	2	3	2.53	.93803	.92072	.011838	.18	3	53.02	54.18	19/68
"	88	10	a	10	1	3	–	.90399	.89152	.016198	.13	2	27.67	72.50	10/77
M. ochrogaster/F	76	50	–	50	1	3	–	.97694	.93082	.011454	.52	–	–	21.18	50/25
"	76	20	c	15	2	2	19.52	.94566	.93207	.011246	.23	4	32.12	69.61	15/60
"	76	40	b	14	2	3	2.74	.94693	.93475	.010803	.11	4	34.99	77.74	14/61
"	76	10	a	10	1	3	–	.93441	.92432	.012530	.17	5	33.72	92.60	10/65
M. ochrogaster/M + F	164	50	–	50	1	3	–	.93290	.90320	.015090	.26	–	–	31.42	50/113
"	164	20	c	19	2	2	20.53	.92181	.91149	.013798	.05	6	51.24	89.35	19/144
"	164	45	b	19	2	3	13.32	.91933	.90869	.014235	.17	6	60.87	86.37	19/144
"	164	10	a	10	1	3	–	.89449	.88759	.017525	.08	7	47.92	129.70	10/153
M. californicus + *ochrogaster* (M + F)	478	10	a	10	1	3	–	.85483	.85172	.019447	.03	23	63.77	274.99	10/467

where it is in fourth row). However, analyses using only the first ten variables selected by the stepwise regression program (the last line of each group in Table 14) show only minor differences in ADJRSQ (≤ 0.02 in most cases) or in RMS (≤ 0.002) compared to the best cases. *Microtus ochrogaster* males and males + females show somewhat higher discrepancies.

C_P-values from different groups should not be compared with each other unless the number of variables is the same; they represent a minimum for a given analysis. According to Chatterjee and Price (1977) it should be close to p (the number of terms in the regression equation). When this is not the case, it is mainly due to the fact that the variance used to estimate C_P is taken from the model with all the variables. If the RMS of the model with all variables is greater than that for a subset with fewer variables, as is the case in our analyses, the C_P-values will be distorted and not be very useful in variable selection.

Cook's distance (Cook, 1977; Dixon and Brown, 1977) is a measure of the change in the coefficients of the regression that would occur if the case were omitted from the computation of the coefficients. In Table 14, only maximal values are given. Cook's distance values are plotted against $\log_{10}$-age for *M. californicus* females (Fig. 13). No correlation with age is evident; only a few cases present high values and can be considered as outliers. For Mahalanobis distances, again only maximum values are given in Table 14. Mahalanobis distances are also plotted against $\log_{10}$-age for *M. californicus* females (Fig. 14). A few points can be considered as outliers, but they are not the same individuals as in Fig. 13.

Because our goal was to predict age using cranial measurements, it was desirable to investigate how far the model fitted the real data and see how the residuals were distributed. Figure 15 represents the predicted age ($\log_{10}$) plotted against $\log_{10}$-age for *M. californicus* females. For ages around 1 month ($=30$ days, $\log_{10} = 1.48$) there were only two serious outliers, but as age increased, the prediction tended to diminish in accuracy. The residuals (predicted-observed values, in $\log_{10}$-units) are plotted against $\log_{10}$-age in Figure 16 for *M. californicus* females. They are normally distributed, but show a significant positive correlation with $\log_{10}$-age ($\alpha < 0.01$). This is also the case for the other subsamples.

A deleted residual is defined as the residual that would be obtained had the case been omitted from the computations of the regression line. If the removal of a case does not change the value of the residual, then by plotting residuals against deleted residuals, as in Figure 17, for *M. californicus* females, we should get a straight line. That is what we observe; there are no serious outliers.

In Figure 18, the studentized residuals are plotted against their expected values for *M. californicus* females. A straight line should be obtained, which is the case, except for the extreme values, both positive and negative. Similar results were obtained for the other subsamples.

It is possible to use the standard error of the estimation (SE), which is the square root of the residual mean square (RMS), to define a confidence

interval for the estimation of age by the regression model. By taking an average standard error of 0.12 (in $\log_{10}$-units) we have the following

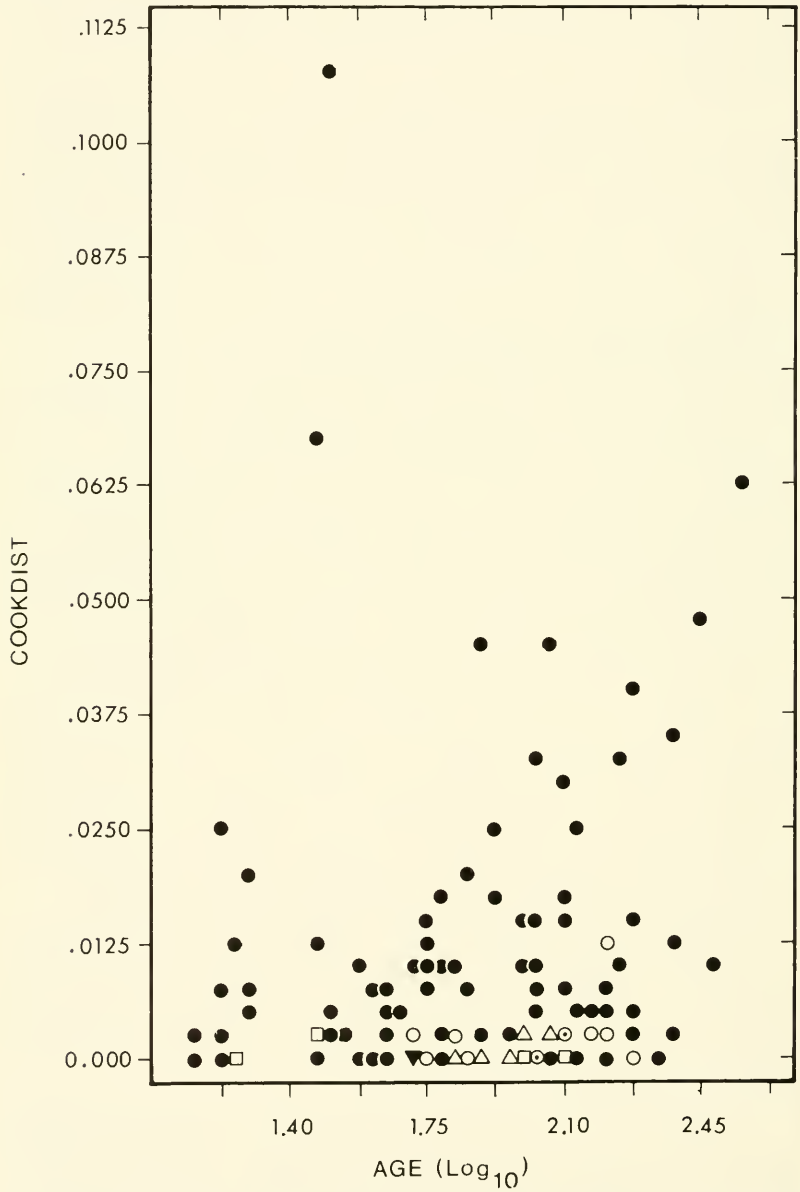

FIGURE 13.—Plot of Cook's distances against $\log_{10}$ age for *M. californicus* females. Results from the multiple regression analysis using program BMDP-9R with the first 10 variables selected by program 2R. Log_{10}-transformation of all variables. Solid circle = 1, open circle = 2, open square = 3, open triangle = 4, solid triangle = 5, dotted circle = 6, solid square = 7, half-solid square = 9.

confidence intervals: 1 month (17-52 days), 2 months (35-104 days), 3 months (52-156 days), 6 months (104-313 days), 12 months (107-626 days). By transforming the logarithmic values into real numbers, two

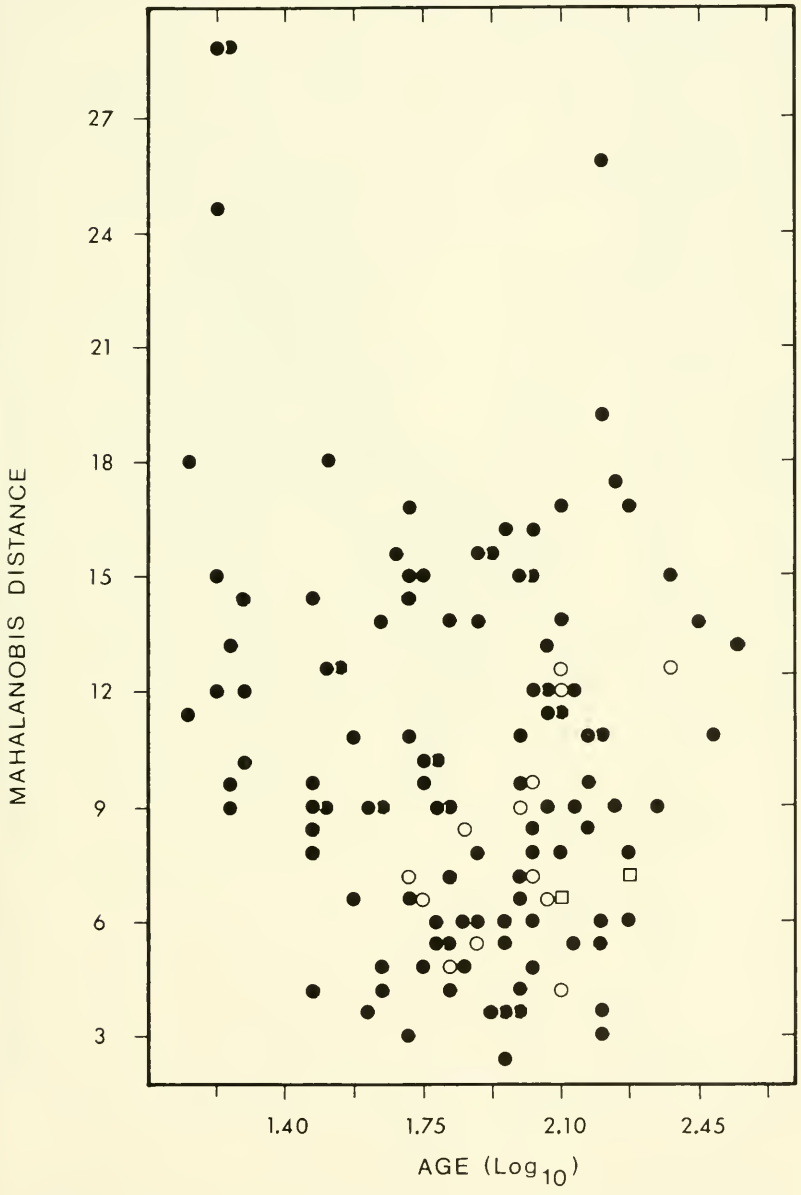

FIGURE 14.—Plot of Mahalanobis distances against $\log_{10}$-age for *M. californicus* females (n = 141). Results from the same analysis, and same symbols, as in Figure 13.

things happen: the confidence intervals become asymmetrical and they increase with age. This is one of the drawbacks of transforming data into logarithms. However, as mentioned above, without a logarithmic transfor-

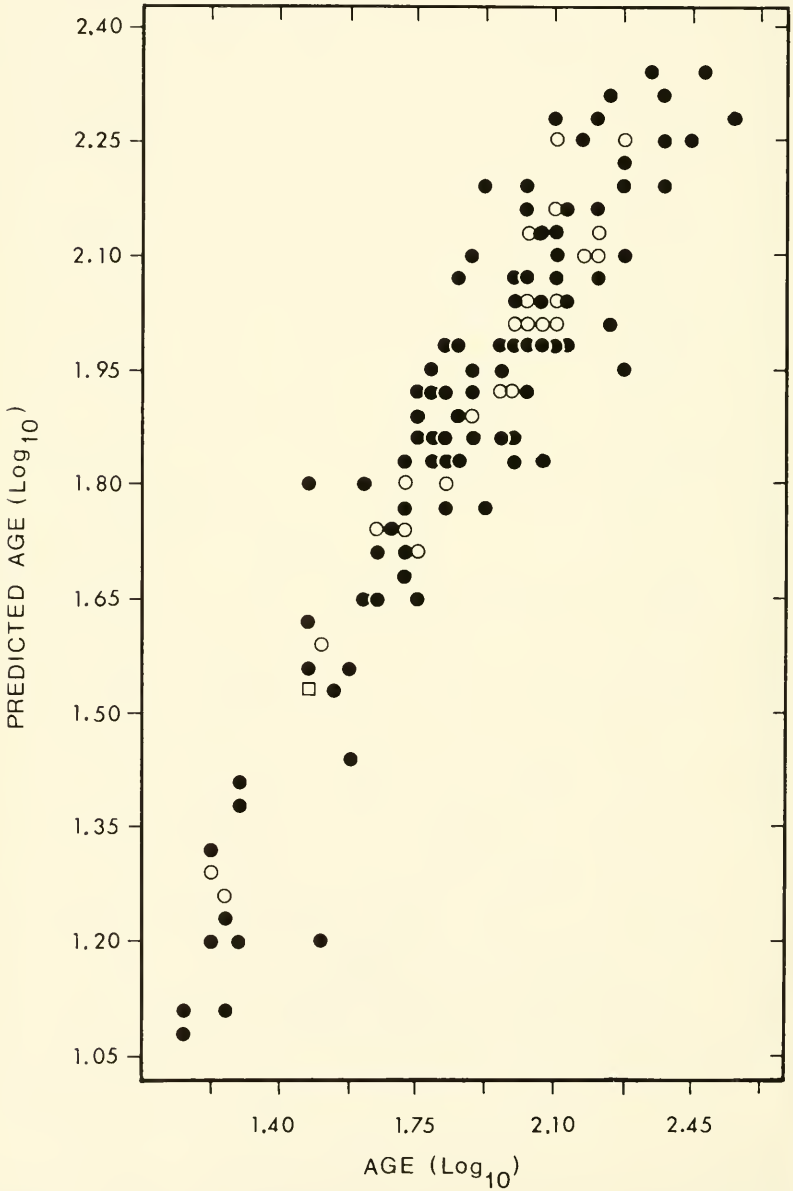

FIGURE 15.—Plot of $\log_{10}$ = predicted age against $\log_{10}$-age for *M. californicus* females (n = 141). Results from the same analysis, and same symbols, as in Figure 13.

mation, we could not have applied a linear model to our data. The logarithmic transformation and the positive correlation of residuals with age are both responsible for the wider confidence intervals as age increases.

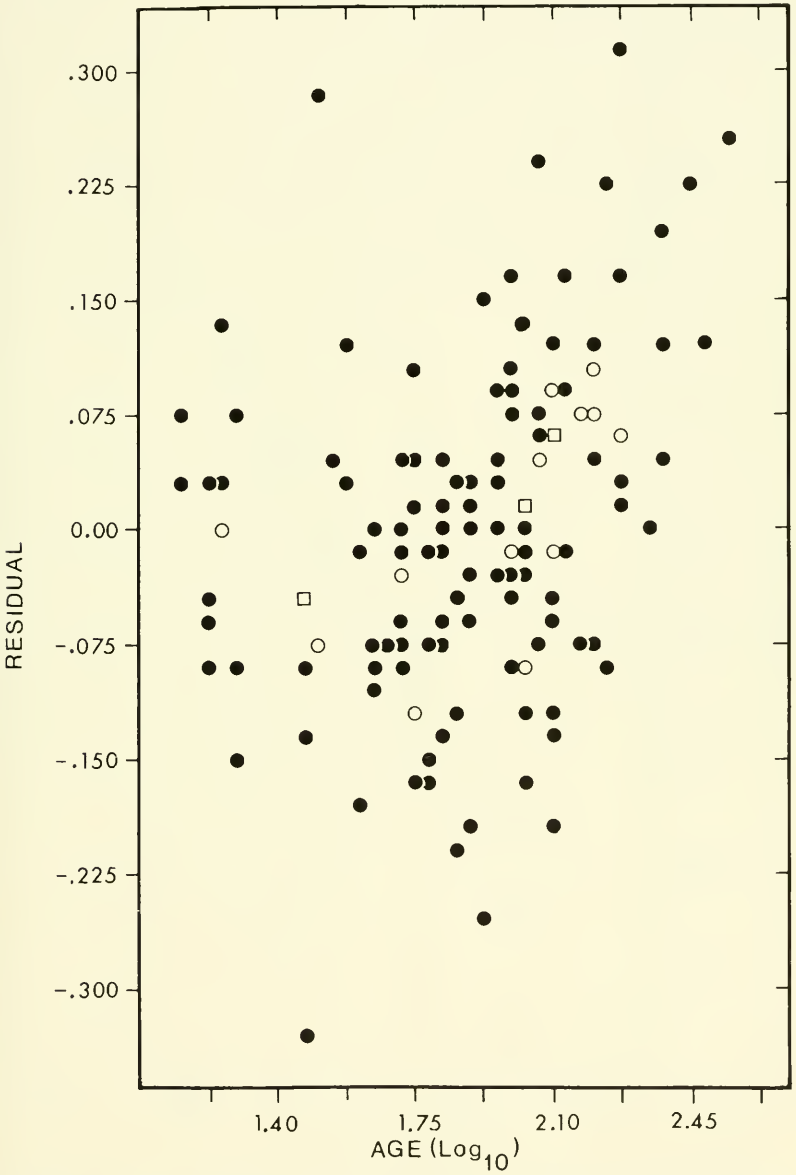

FIGURE 16.—Plot of residuals against $\log_{10}$-age for *M. californicus* females (n = 141). Results from the same analysis, and same symbols, as in Figure 13.

A comparison of our results with those given by Lidicker and MacLean (1969) is difficult because of their division of the sample into two groups: individuals less than and more than 100 days old. We can,

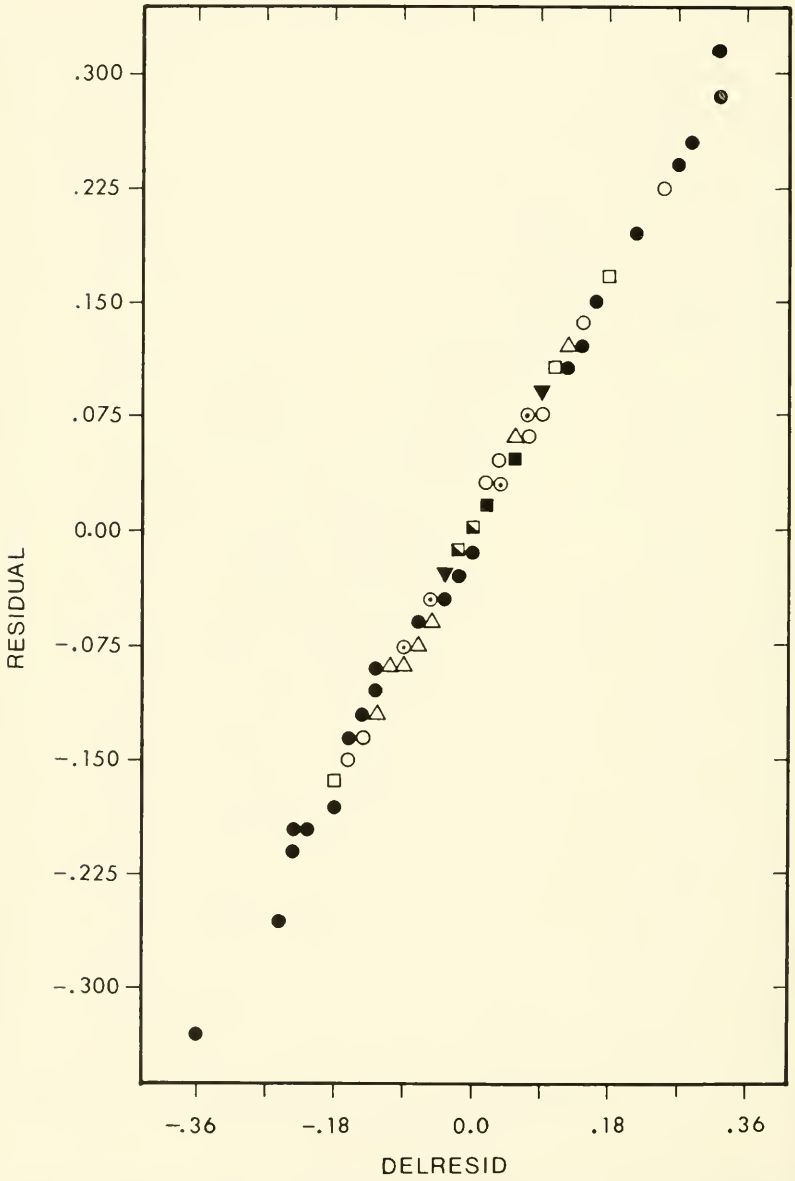

FIGURE 17.—Plot of residuals against deleted residuals for *M. californicus* females (n = 141). Results from the same analysis, and same symbols, as in Figure 13.

however, compare our two month-values with the less-than-100-days old and the 6 month-values with the over-100 days old. We see then that our values for the confidence intervals are higher than those reported by Lidicker and MacLean (1969) for both of the methods they described and

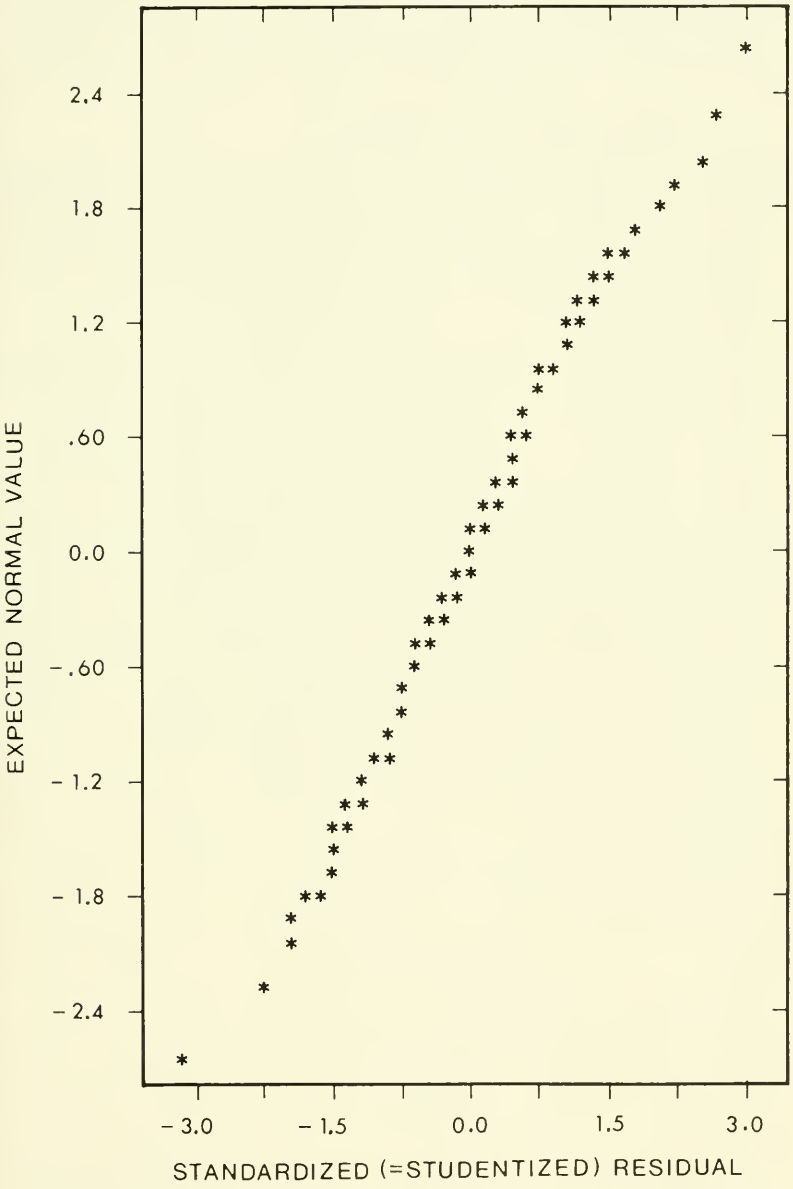

FIGURE 18.—Plot of expected normal values against standardized (studentized) residuals for *M. californicus* females (n = 141). Results from the same analysis as in Figure 13.

in both age-classes, although those for the growth curve method are nearly as high as ours for the older specimens. The usefulness or appropriateness of either model is debatable. Ours encompasses all specimens up to one year, but needs more variables and a logarithmic transformation of the data, whereas the approach by Lidicker and MacLean (1969) has the advantage of using fewer variables, without a logarithmic transformation, and gives somewhat more accurate results, but is more cumbersome to use.

DISCUSSION

Analysis of repeated measurements (2-way ANOVA) indicated that for most variables, observed discrepancies were not statistically significant; only in two cases was there a significant difference. This should give morphometrists confidence in cranial measurements. It is, however, worth stressing that care should be taken in defining and describing measurements.

The coefficient of variation (CV) measures the relative error compared to the mean. Thus a large measurement will have a lower CV than a smaller one with the same standard deviation. By defining an expected CV over the whole measuring range based on a given standard deviation which is assumed to be the same for all the measurements, it was possible to compare the different variables with each other through their CV-values (see Figure 4). Most good discriminators between the two species, such as B4, B6, B12, B14, H5 show a lower CV than expected, L16 being an exception. The best discriminators between sexes (L9, L14, B4, B13, B14 for *M. ochrogaster* and L8, B5, B11, H2 for *M. californicus*) are rather close to the expected values, although L14 and H2 have higher CVs and B4 and B14 lower ones. Good age indicators are to be found both above (L13, L16, H3) and below (B3) the curve of expected values. It is difficult by inspection of Figure 4 to select variables for further analyses. Variables which would have been discarded because of high CV are the most useful in species or sex discrimination or age estimation, L16 being a good example. In cases where two or more variables are highly correlated with each other, as for instance, L1, L2, L3, L4, L5, or B3, it would be advisable to take those with the lowest CV. For these variables, however, the differences are only minor, and any of them could be chosen. Moreover, some measurements are easier to take or do not need special calipers, so that eventually several factors have to be considered when selecting a set of variables to be measured.

In PC-analysis performed on both species taken together, the first PC accounts for approximately 65 percent and the second about 13 percent of the total variation. Their interpretation is somewhat difficult because each of them includes different components of variation. It is not possible in this case to conclude, as many authors have done in other species, that factor 1 is a size factor only and the other components are shape vectors. Oxnard (1978) warns against a too simplistic interpretation of principal components in terms of size and shape. Furthermore, both age and species

components in size variation are being studied here. Unless both groups overlap in the multivariate space, it will not be possible to fit axes accounting for size, age or species variation only. Each component will be of a mixed nature. Possibly, by rotation of axes, as in factor analysis, it would be possible to maximize (or minimize) variation on the different components considered. A PC-analysis performed on each species separately showed that the first factors of each had different directions, the angle between them being about 22° ($\cos\Theta = 0.928$).

An initial step in many multivariate data analyses is PC-analysis in order to detect groups. We have shown, in a situation where two groups were already well defined, that interpreting PC axes as simple size and shape vectors was hazardous. The goal of PC-analysis is to extract components of variation, reducing the whole set of variables to a few components accounting for as much variation as possible, and usually easier to interpret. In our study, factor 1 accounts mainly for age-related size variation and factor 2 for the interspecific differences (Figure 5). By taking each species separately, the results are somewhat clearer, factor 1 being the only component highly correlated with age, but the other components, especially factor 2, while more difficult to interpret, carry information about sexual variation.

In DF-analysis, age variation can mask other sources of variation, mainly that variation due to taxonomic differences. Naturally this is considered a major problem by systematists and explains why animals are usually assigned to different age classes which are then analyzed separately. In our case, age variation does not play too important a role when discriminating between species, perhaps because interspecific variation is of a different character than age variation, the former being mainly due to shape differences and the latter to size differences. In other cases in which interspecific and age variation are similar, it might be useful to remove the effect of age. Burnaby (1966) has proposed growth invariant discriminant functions. Vectors correcting for the factors whose effects we wish to eliminate must first be estimated; one way to make such a correction might consist of taking factor 1 from a PC-analysis and consider it as a growth factor (Jolicoeur, 1963). However, as we have pointed out, factor 1 from an analysis performed on each species taken separately should be used, rather than from a PC-analysis computed from both species together. In each species, factor 1 is highly correlated with age ($r = 0.9$) and accounts for approximately 50% of the total age variation.

Canonical correlation analysis is a parsimonious way to express relationships between variables. In our case, we have three sets of variables—lengths, widths and heights—which are perpendicular to each other, but not uncorrelated. Canonical variates are orthogonal (uncorrelated) within the same set, and the loadings on them for the several variables considered allow us to find out which variables are mainly size or age related. Paired comparison between the different sets showed a high correlation between the canonical variates; *i.e.*, the different sets of variables carry similar information. In the comparison of width versus

length, variables which determine different shapes such as B4, B6, B11, VB12, B13, L10, L14, L15, L16, L24 become apparent in the second pair of canonical variates. For example, in *M. ochrogaster* wide interorbitals (B4) is correlated with wide incisors (B13), while in *M. californicus* the interorbital is narrow, and the incisors also narrow. Different shapes are determined by the relative size of these measurements. By plotting the second canonical variates of each set against each other two groups which correspond to the species appeared (Figure 10). A canonical correlation analysis using more than two sets of variables (Horst, 1961; Kettenring, 1971) could be used to perform a simultaneous comparison of height, width and length measurements.

Canonical correlation has been used to relate morphological to climatic data (Boyce, 1978) or morphological variables from different parts of the body (Johnston, 1976), but not, to our knowledge, to compare different skull variables. We think that an approach along that line would reveal interesting relationships between variables, leading to a better understanding of differences in shape between taxa.

Both programs used in multiple regresssion analysis (best subset (BMDP-9R) and stepwise regression (BMDP-2R)) gave similar results (Figures 11 and 12). With the former, one is sure that no variable has been overlooked, because all relevant combinations are tried. In the stepwise procedure it may happen that a variable entered at the beginning of the analysis is not the best one when other variables are also included in the equation. However, in such cases, it is often removed in a later step and replaced by a more suitable one. One decisive advantage of program BMDP-9R resides in the availability of Cook's and Mahalanobis distances, studentized residuals, and deleted residuals, which allow a thorough analysis of residuals and outliers. In most cases, the first variable entered in the analysis accounts for approximately 80 percent of age variation. To get another 10 percent it is necessary to include up to 10 variables. Thus, most variables used in our study were redundant, and once a variable which is highly correlated with age is selected, any other variable makes only a meager contribution to explain age variation. It is probably impossible to find skull variables whose combination gives a better fit. Our results can be considered as a limit and there will always remain around 10 to 15% of total age variation which cannot be explained with cranial measurements.

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advice. Deb Bennett drafted the figures, and Jan Elder typed the final drafts.

SUMMARY

A morphometric analysis of 314 specimens of *Microtus californicus* and 164 of *M. ochrogaster* reared in the laboratory was conducted using 47 skull measurements, cranial and mandibular weights and head + body length.

Repeated measurements performed on a separate sample of *M. ochrogaster* (n = 10) were used to estimate the measuring error through a 2-way analysis of variance. Nearly all variables can be considered as reliable when defined correctly.

Factor 1 from a principal components analysis performed on both species combined is highly age correlated and accounts for approximately 30 percent of total age variation. Factor 2, though also age correlated, accounts mainly for interspecific difference. The first factors from analyses on each species separately account for about 50 percent of total age variation whereas second factors are age independent and account for much of the differences between sexes.

Discrimination between the species improved with increasing age of specimens. Sexual dimorphism is not very pronounced in either species. Mandibular measurements separate the species and the sexes less well than the cranial variables.

Canonical correlation analysis showed that length, width, height, cranial and mandibular measurements convey similar information about the skull. Second canonical variates derived from the comparison between length and width measurements separate the species well and allow a characterization of shape for each group through the interpretation of the loadings on the canonical variates.

Multiple regression analysis was used to predict age from skull measurements. A $\log_{10}$-transformation was performed to linearize the data. About 85% of age variation can be accounted for by a model with 3 variables and 90% with one comprising 10 variables. Many variables used here are highly correlated and therefore not needed for age prediction.

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