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MICROGEOGRAPHIC MORPHOLOGICAL DIFFERENTIATION IN MUSKRATS

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Cranial morphometry was studied in local populations of muskrats inhabiting ponds along various tributaries in a single river drainage (150 km^2) in southern Belgium. Despite the absence of noticeable environmental heterogeneity across this region, significant morphological differences were found among local populations. This differentiation was not related to gender, and it remained after controlling for the age and size components of variation. Spatial analysis confirmed the existence of a microgeographic pattern of differentiation, which is related to variation in shape of skull. Isolation by distance along corridors is proposed as a mechanism to account for the observed pattern of differentiation. This mechanism involves population isolation, genetic drift, and a small amount of socially-induced gene flow among local populations that mainly are connected to one another through the river network. According to this model, the distance between local populations is suitably represented by the number of "decisions" a muskrat must make when travelling along the course of the river from one tributary to another. Data are consistent with 10 quantitative predictions derived from the proposed model involving morphological, straight-line, and "decision" distances among populations.

Key words: *Ondatra zibethicus,* muskrat, Mantel test, morphometry, skull

Individuals of sexually reproducing species differ phenotypically and genetically. A detailed study of the sources of differentiation among intraspecific groups of individuals may provide clues concerning mechanisms of microevolution (Gould and Johnston, 1972; Smith et al., 1978). Differentiation may be assessed based on nuclear or mitochondrial DNA, or through phenotypic manifestations such as allozymes or epigenetic variants, or morphometric variation (Bauchau, 1988; Jones et al., 1980). Morphology has the advantage of being easy to measure and polygenically controlled (Atchley, 1983; Thorpe, 1983), and it may be highly heritable (Atchley, 1983), although not necessarily so (Cheverud, 1982). For instance, morphometric measures on the mandible suffice to discriminate among genetic strains of house mice (Festing, 1972). However, morphology is subject to environmental influences (Smith et al., 1978; Straney and Patton, 1980); but in muskrats, environmental conditions have been shown to affect size rather than shape of skull (Boyce, 1978; Pankakoski, 1983; Ruprecht, 1974). Pankakoski and Nurmi (1986) attributed differences in shape of skull to genetic, rather than to environmental factors.

Intraspecific phenotypic variation is well documented among widely distant populations for a variety of organisms, including muskrats in North America (Boyce, 1978) and in Europe where they have been introduced (Pankakoski and Nurmi, 1986; Ruprecht, 1974). Such differentiation often has been interpreted as being of environmental origin (Boyce, 1978, Straney and Patton, 1980), and more rarely of genetic origin

(Bowen, 1982; Chesser, 1983; Smith et al., 1978). In small mammals in particular, intraspecific morphometric differentiation has been related to latitude (Baker et al., 1978), geographic barriers (Smith and Patton, 1984), or density cycles (Bowen, 1982; Mihok and Fuller, 1981). Nonetheless, the problem when dealing with widely distant populations is that genetic and environmental factors easily confound phenotypic differentiation (Dillon, 1984; Jones et al., 1980; Pankakoski, 1983). Thus, Rohlf and Schnell (1971) warned of the difficulty of distinguishing variation in phenotypes produced by environmental gradients from that produced via isolation by distance.

At microgeographic scale, however, habitat heterogeneity may be absent, or at least strongly reduced, in which case phenotypic differentiation should be caused by genetic, rather than by environmental factors. But the literature contains contradictory findings on neighboring populations of small mammals. Some studies do not reveal interpopulation differentiation (on morphometry and allozymes—Tolliver et al., 1987; on epigenetic variants—Sikorski and Bernshtein, 1984). The authors explain this by gene flow between neighboring populations. In contrast, others find differentiation (Chesser, 1983; Pankakoski, 1983; Patton and Feder, 1981) and propose some form of isolation of populations.

Indeed, isolation of populations such as provoked by physiogeographic barriers, or by social factors such as territoriality, philopatry, or differential dispersal (Krebs and Myers, 1974; Patton and Feder, 1981), may induce phenotypic differentiation, especially when coupled with severe reductions in population size (bottlenecks—Bauchau and Le Boulengé, 1991; Bowen, 1982; founder effect—Berry, 1986) that enhance genetic drift. Nonetheless, isolation of populations does not necessarily lead to phenotypic differentiation (Ehrlich and Raven, 1969), and drift alone should produce variation that is independent of the geographic location of a population (Dillon, 1984). Drift is unlikely to produce geographic gradients.

We take advantage of a situation particularly well-suited for studying sources of phenotypic differentiation. During an extensive trapping campaign, we observed that local populations of muskrats (*Ondatra zibethicus*) from throughout the drainage basin of a small river in southern Belgium were differentiated in cranial morphology. Although muskrats form relatively isolated micropopulations in this area, inhabiting ponds scattered along the affluents of the river (Le Boulengé, 1974), the environmental conditions over the whole area seem too homogeneous to have induced phenotypic differentiation in such closely neighboring populations. Hence, the general question addressed in this paper is how and why are these local populations of muskrats differentiated?

We first establish that the local populations of muskrats are differentiated morphologically, and describe the contribution of cranial characteristics to this differentiation in terms of components of age, size, and shape. Next, we investigate the spatial pattern of morphometric variability. Because the study area is small and largely homogenous, observed variation in structure of the skull is likely to have a genetic rather than environmental basis. Variability in size, which is the most probable effect of diet on cranial morphology (Pankakoski, 1983), can be removed from the data. In contrast, genetic drift likely occurs in local populations of muskrats, because they are subject to important declines in spring, which are under the control of social mechanisms such as competition among adults for territories (Errington, 1963; Le Boulengé and Le Boulengé-Nguyen, 1981). A sociobiological mechanism called isolation by distance along corridors is proposed to account for the observed variation. Predictions of this model are tested with morphologic and geographic distances among local populations of muskrats.

MATERIALS AND METHODS

Study area.—The study area covers the 150 km^2 drainage basin of the La Houille River, from the springs near the headwaters to its confluence with its main tributary, La Hulle (Fig. 1). This corresponds to the upper and middle courses of this small river in the Ardennes of southern Belgium. About 350 ponds are scattered along the tributaries, forming the bulk of the region's suitable habitat for muskrats. These ponds generally are small (mostly <1 ha, maximum 9 ha) and manmade for angling. Along its lower course in France, below its confluence with the La Hulle River, the River drains few tributaries and these have almost no ponds. It joins the La Meuse River in Givet, France, ca. 10 km NW of Felenne (Fig. 1; Le Boulengé, 1974). This drainage basin occurs on schistose Gedinnian substrate (Duvigneaud, 1972) within a single bioclimatic and phytogeographic area, the Ardennian-Eifelian subdistrict (Anonymous, 1972). In general, the valleys of the tributaries are steep and separated by forested heights of the Ardennes Plateau (shaded areas in Fig. 1). Duvigneaud (1972) describes the strong elevational gradient of vegetation that occurs along one of these side valleys. Thus, differences of vegetation or physical environment along the course of the main river are not greater than within valleys of tributaries. The River generally is 5–10 m wide, whereas tributaries often are <0.5 m wide and do not offer muskrats an opportunity to swim.

Geographic origin and population zones.—Muskrats caught during an intensive trapping campaign in ponds of the drainage basin of the La Houille River between October 1971 and February 1972 were used in this study. Our study followed the capture-recapture study reported in Le Boulengé and Le Boulengé-Nguyen (1981). A total of 493 muskrats were trapped during this campaign. A population of river-dwelling muskrats occurred in the La Houille River, but was excluded from the present analysis because it did not correspond to a single, clearly distinct local population (Le Boulengé and Le Boulengé-Nguyen, 1981). After excluding animals with broken skulls, damaged eye lenses, or without date and locational data, the usable sample of pond-dwelling muskrats totalled 144 individuals (70 males, 73 females, and one damaged, unsexed individual; Table 1).

We defined a local population as the set of muskrats caught in the drainage basin of a single tributary of the La Houille River (Fig. 1); we called the corresponding geographic area a population zone or zone. Almost all captures occurred in ponds, although traps also were set in tributaries themselves; population zones were thus clearly distinct geographically, and in most instances separated from the population of river-dwelling muskrats by uninhabited stretches of tributary. Also, the population zones generally were surrounded by dense coniferous plantations or mixed oak-beech (*Quercus-Fagus*) stands with steep slopes. These forests acted as barriers isolating the population zones from one another; dispersal of muskrats, thus, mainly followed the water courses (Le Boulengé and Le Boulengé-Nguyen, 1981).

Two of the zones, M and Z (Fig. 1), did not follow this general pattern, as their upper parts were only separated from each other, and zone M from the upper part of zone C, by a short strip of swampy area (white in Fig. 1). Hence, muskrats could move directly between these zones. In support of this view, a muskrat released in the River near zone E was recaptured a few days later in the village of Rienne, between zones C and M. Thus, any process at work for isolated populations may follow a different pattern in these less-isolated zones. For this reason, local populations M and Z were not included in analyses of the spatial pattern of morphometric differentiation.

To study the spatial pattern of morphometric differentiation, two types of distances were calculated between all pairs of population zones (Tables 2 and 3); straight-line and along water courses. The latter was partitioned into two segments; the distance along the main river (between entry points to the respective side valleys), representing the swimming distance, and the other along the tributaries (from the entry point to the side valley, or confluence of the tributary with the main river, to the center of the zone), representing the walking distance. Centers of zones were defined as the arithmetic mean coordinates of the locations where usable muskrats had been captured. Finally, a third type of distance, called decision distance, was defined in the framework of our model of isolation by distance along corridors (below).

Characters and measurements.—For each captured individual, gender was determined by inspection of the genitalia after dissection (Le Boulengé and Le Boulengé-Nguyen, 1981), and

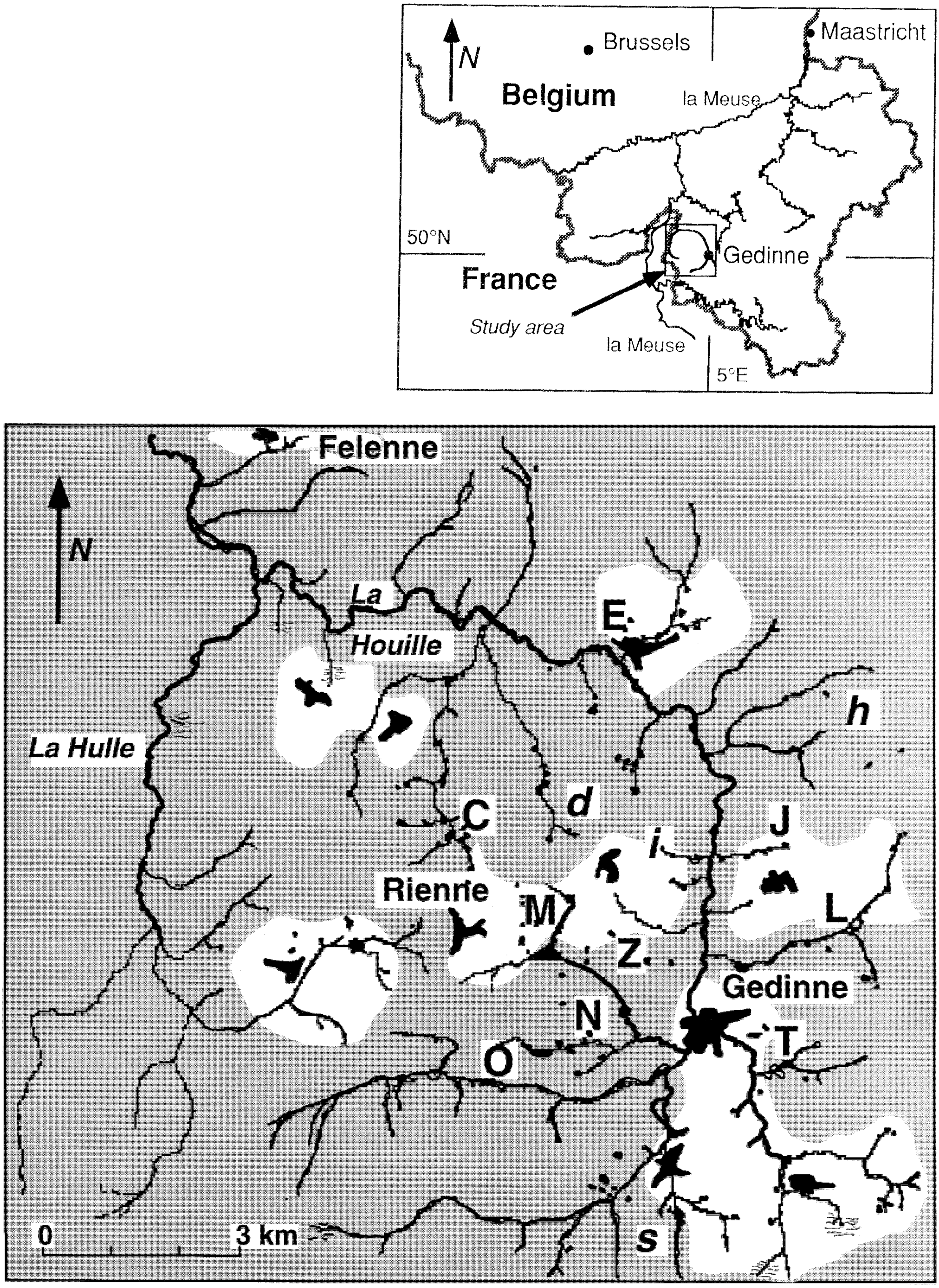

FIG. 1.—Drainage basin of the La Houille River, showing tributaries, ponds, marshy areas, villages, and 13 population zones inhabited by muskrats. Zones are identified by letters (uppercase: zones included in the present study; see Table 1). The nine zones used in this study, yielding at least five usable muskrats each, are C, E, J, L, M, N, O, T, Z. White areas are non-wooded (swamps, meadows, and cultivated fields), and shaded areas are forests. The insert shows the location of the study area within the broader hydrogeographic system of southern Belgium.

TABLE 1.—*Samples of muskrats included in the study*[a].

Zone	C	E	J	L	M	N	O	T	Z	Total
Females	3	5	4	24	21	3	5	5	3	73
Males	7	5	4	18	16	3	4	9	4	70
Undetermined				1						1
Total for the zone	10	10	8	43	37	6	9	14	7	144

[a] Geographic zones are shown in Fig. 1.

age was estimated from mass of eye lenses using the technique and growth curve given by Le Boulengé (1977). Fifteen linear measurements were taken on each skull, seven on the mandible (those measurable with the highest precision; Fig. 2) and eight on the cranium (same measurements and abbreviations as in Pankakoski and Nurmi, 1986:figure 2 and Ruprecht, 1974: figure 1, except where indicated): CL, condylobasal length; DL, length of diastema (our measure includes the incisor); UML, length of maxillary toothrow; BL, length of braincase (our measure excludes UML); RW, width of rostrum; ZW, zygomatic breadth; IW, interorbital constriction; BW, width of braincase. Measurements were made with digitalizing dial callipers (Digibit of Numonics Corporation, equipped with a home-made aiming device that corrects for parallax; resolution, 0.25 mm) connected to an Altos ACS-800 microcomputer. All bilateral measurements were obtained from the left side of the skull, to avoid potential problems related to asymmetry (although directional asymmetry is improbable—Leamy, 1984). Skulls were processed in a random order with respect to zones.

Morphological differentiation of populations.—The significance of morphological differences among local populations was assessed using univariate analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA). The observed differences were divided into components of age, size, and shape for the study of spatial patterns. This required particular adaptations of principal-component and regression-analysis techniques; these steps are detailed in Appendix I. The SAS codes (SAS Institute, Inc., 1989) implementing them may be obtained from the first author. Datasets resulting from these preliminary analyses are presented in Fig. 3. Mean pairwise morphological differences among population zones were quantified as Mahalanobis distances.

Spatial patterns of morphological variation.—The Mantel test (Mantel, 1967) was used to determine if Mahalanobis distances among local populations of muskrats were correlated with spatial distances (Dillon, 1984; Legendre and Fortin, 1989; Sokal, 1979). Spatial patterns of morphometric characteristics were studied by multivariate Mantel correlogram analyses (Sokal et al., 1987). Spatial distances among zones were first reduced to a series of classes; for each class, a spatial weight matrix was constructed, in which all pairs of zones falling within that distance class were represented by 0, and all other pairs by 1. Each spatial weight matrix was

TABLE 2.—*Straight-line (in kilometers, above the diagonal) and decision (below the diagonal) distances among population zones*[a].

Zone	C	E	J	L	M	N	O	T	Z
C		3.79	5.20	6.39	2.25	3.77	3.95	6.14	3.70
E	2		4.04	5.56	4.41	6.10	7.00	6.79	4.81
J	4	3		1.52	3.80	4.46	5.90	3.28	2.72
L	5	4	2		4.58	4.74	6.23	2.49	3.14
M	7	6	4	3		1.76	2.59	3.93	1.56
N	7	6	4	3	1		1.49	3.13	1.75
O	8	7	5	4	2	2		4.47	3.20
T	6	5	3	2	2	2	3		2.44
Z	5	4	2	1	3	3	4	2	

[a] Geographic zones are shown in Fig. 1.

TABLE 3.—*Swimming (along the main river, above the diagonal) and walking (along the tributaries, below the diagonal) distances among population zones*[a], *in kilometers. The total distance along the waterway is the sum of the swimming and walking distances.*

Zone	C	E	J	L	M	N	O	T	Z
C		2.10	6.50	8.13	10.75	10.75	10.38	9.50	8.13
E	3.43		4.40	6.03	8.65	8.65	8.28	7.40	6.13
J	4.55	1.13		1.63	4.25	4.25	3.88	3.00	1.63
L	5.95	2.53	3.65		2.63	2.63	2.25	1.38	0.00
M	5.75	2.25	3.25	4.88		0.00	1.38	1.25	2.63
N	4.63	1.20	2.33	3.73	3.25		1.38	1.25	2.63
O	6.25	2.83	3.95	5.35	5.75	3.78		0.88	2.25
T	4.53	1.10	2.23	3.63	3.88	2.30	3.93		1.38
Z	4.50	1.00	2.00	3.63	3.25	2.00	4.50	2.63	

[a] Geographic zones are shown in Fig. 1.

then compared to the matrix of Mahalanobis distances among zones, by computing the standardized Mantel statistic. The correlogram was obtained by plotting the values of the Mantel statistic (ordinate) as a function of the spatial distance classes (abscissa).

Finally, we used the method of Smouse et al. (1986) for testing the association between two distance matrices, which remains after controlling for the distances in a third matrix. The partial Mantel statistic was computed using the formula of the partial Pearson correlation coefficient, but its significance was tested through the random-permutation method. We generally based our tests on 1,000 pseudorandom permutations, except for the correlograms, or when the observed probabilities approached the significance level. In these instances, as advised by Jackson and Somers (1989), we used 10,000 permutations. Simple and partial Mantel tests and correlograms were computed using "The R Package" (Legendre and Vaudor, 1991).

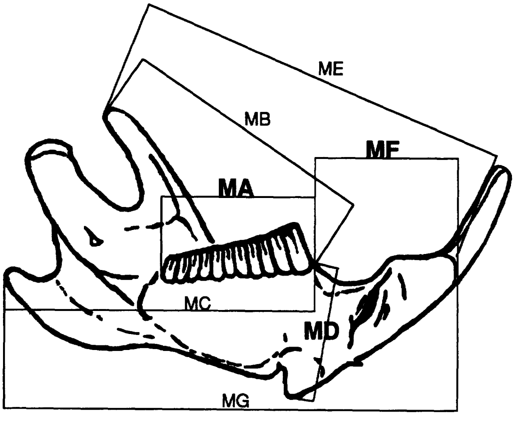

FIG. 2.—Seven measurements taken on the left mandible of muskrats. MA, length of mandibular toothrow (LML in Pankakoski and Nurmi, 1986:figure 2); MB, mandibular shape B; MC, mandibular length C ((ML5) in Pankakoski and Nurmi, 1986:ML5 minus diastema); MD, mandibular height D; ME, mandibular shape E ((ML2) in Pankakoski and Nurmi, 1986); MF, mandibular shape F (shortest length of diastema); MG, mandibular length G (ML5 in Pankakoski and Nurmi, 1986).

Isolation by distance along corridors.—Genetic, and consequent morphological differentiation, can be produced by a mechanism of isolation by distance along waterways, or more generally along corridors. It is related to the well-known isolation-by-distance model (Rohlf and Schnell, 1971; Wright, 1943) and is based upon a number of behavioral characteristics of muskrats. Muskrats form relatively isolated local populations in the study area, for four reasons: first, centers of side valleys are separated from the main river by often torrential stretches of creek passing through forested areas that are unsafe for muskrats (Le Boulengé, 1974); second, side valleys are separated from one another by unfavorable forested crests (except between zones Z and M, and between M and upper C); third, reproductive males are territorial during spring, and females are territorial during spring and throughout the reproduction period (Errington, 1940; Le Boulengé and Le Boulengé-Nguyen, 1981). Thus, successful immigration is uncommon during these periods; finally, young muskrats, after weaning, preferentially settle as close as possible to the home range of their

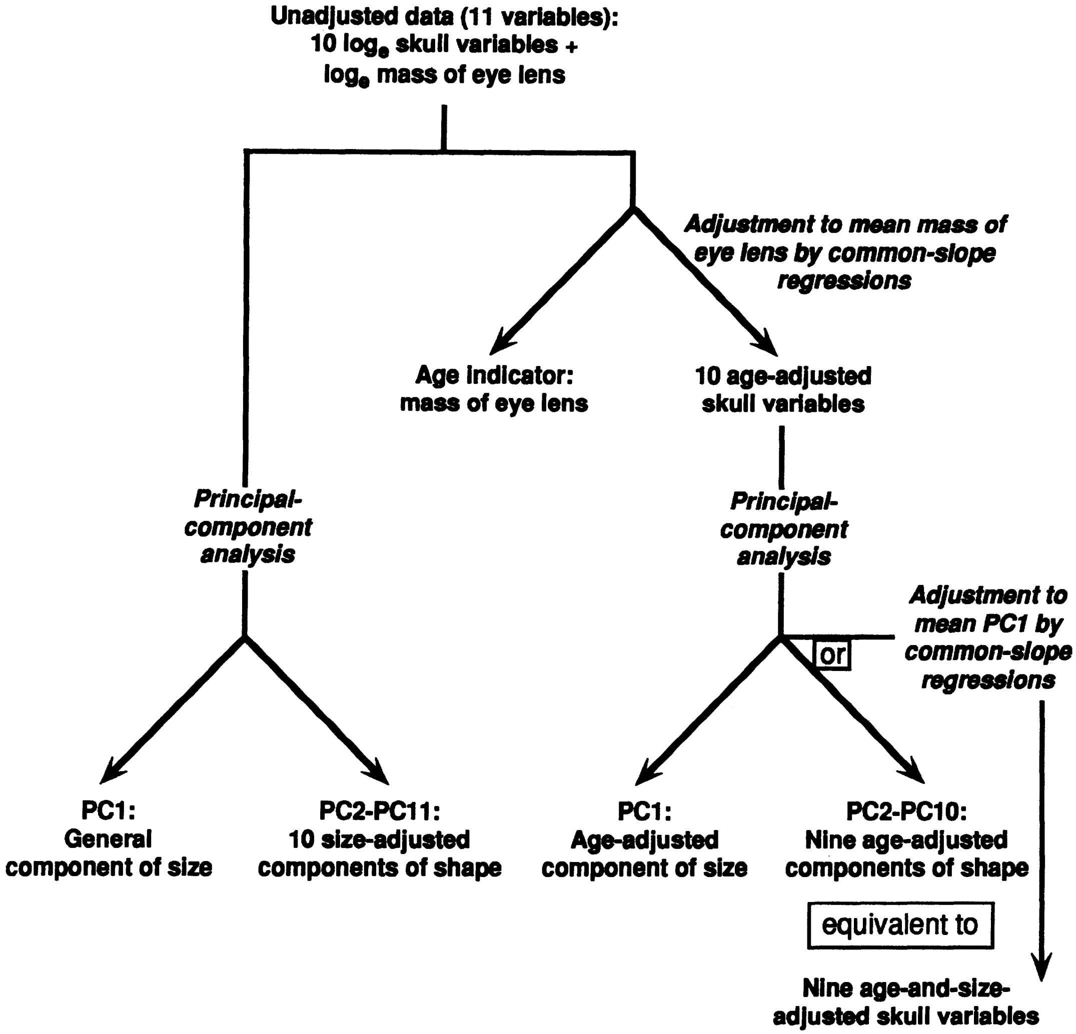

FIG. 3.—Summary of the partitioning of morphometric variables, described in Appendix I. For each dataset, a Mahalanobis distance matrix among zones was computed. The same names are used in the text for data and distance matrices.

mother (Caley, 1987; Le Boulengé and Le Boulengé-Nguyen, 1981). Dispersal among populations occurs, especially by subadults. Populations may incorporate dispersers when territorial behavior relaxes at the end of the reproductive period (Errington, 1940; Le Boulengé and Le Boulengé-Nguyen, 1981). Our model assumes that the probability of successful immigration decreases as distance increases, or more precisely, as the difficulty of moving from one zone to another increases. Difficulty of immigration may be assessed best by some other measure than by straight geographic distance (Dillon, 1984; Jones et al., 1980), perhaps in terms of social, rather than physical, resistance to movement. Muskrats can disperse fast; they are capable of quickly moving long distances. During his study of population dynamics along this River, Le Boulengé (1974) observed a record 10-km-downstream movement by a muskrat in 48 h. Errington (1940) and Mallach (1971) also reported examples of fast movements between distant locations. Dispersing muskrats do not swim directly up or down along a waterway. They intensively explore the banks and immediate surroundings, running on the banks as much as they swim, and

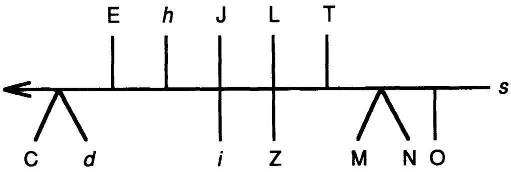

FIG. 4.—Diagrammatic representation of the drainage basin of the La Houille River, where the basins of tributaries are figured as stems rooted on a common trunk standing for the main course of the river. This diagram is used to compute decision distances among zones. All 13 zones represented were inhabited by muskrats, but only the nine zones represented in uppercase yielded sufficient samples ($n \geq 5$), to be used in analyses.

frequently backtracking. Such movement patterns by a migrating muskrat were followed for >2 km in early spring 1971 (Le Boulengé, 1974). To a dispersing muskrat, in a surrounding like the valley of the La Houille River, potentially favorable places to be explored for eventual settlement are either the River itself, or the ponds scattered along tributaries in side valleys. Tributaries themselves do not represent suitable habitats (Le Boulengé, 1974).

We thus view each confluence of the River with a tributary as a point where a dispersing muskrat must make a decision; either to leave the main river and explore the side valley of the tributary, or to proceed along the main river. The number of confluences may thus be taken as an index of dispersal distance among zones, regardless of the actual distance. We call this the decision distance among zones. When evaluating the decision distance between two zones, only those tributaries have been taken into account where muskrats actually were caught, or where signs of recent presence of muskrats were observed (Fig. 4). We assume that tributaries not fulfilling this condition are unsuitable habitat for muskrats. It is possible (but not necessary for our argument) that muskrats are able to perceive this unsuitability without actually having to explore such side valleys. When two tributaries join close to their confluence with the River (as C and d; Fig. 1), or when two tributaries join the River opposite to each other or almost so (as i and J, L and Z; Fig. 1), we counted only one decision (Table 2, below the diagonal).

The above model and arguments imply that morphometric distances among populations (assembled in a matrix called *C*) should be more closely related to decision (matrix *B*) than to straight-line (matrix *A*) distances, which represent most other models of spatial-dispersion mechanisms. The La Houille River is especially well-suited to test this hypothesis, because of its peculiar shape: the River forms a semicircle around the central plateau (Fig. 1), so that decision distances are not strongly correlated to straight-line distances among population zones. If the pattern of morphological differentiation in the local populations of muskrats is, indeed, produced by the processes underlying the above model, the relationships among these three distance matrices should be that *C* depends upon *B*, *B* depends upon *A*, but *C* does not depend upon *A*.

According to Legendre and Troussellier (1988), a series of consequences should follow, concerning the significance of the simple and partial Mantel correlation statistics computed among matrices *A*, *B*, and *C*. These consequences can be verified to test how well the model fits the data. Notation: For example, *AB* represents the correlation between matrices *A* and *B*, while $AB{\cdot}C$ is the partial correlation of *A* and *B* when controlling for the effect of distance matrix *C*; significance is evaluated using the Mantel test: 1) the correlation measured between *A* and *C* should only be the result of the correlations *AB* and *BC*, so the correlation values should be such that $AB \times BC \approx AC$. A consequence is that $|AB| \geq |AC|$ and $|BC| \geq |AC|$; 2) for the model to hold, the simple correlations *AB* and *BC*, at least, should be significant; 3) partial correlations—for the model to hold, it is necessary for $AB{\cdot}C$ and $BC{\cdot}A$ to be significantly different from zero, and for $AC{\cdot}B$ not to be significantly different from zero. It can be shown that the relations $|AB{\cdot}C| \leq |AB|$ and $|BC{\cdot}A| \leq |BC|$ should then hold. Other models of interrelationships among these three distance matrices entail a different set of predictions (Legendre, 1993; Legendre and Troussellier, 1988); hence if the data are consistent with these 10 predictions, the model is supported against alternative models.

RESULTS

Morphometric differentiation.—Of the 15 measured characters, five were eliminated from analyses. Condylobasal length and mandibular shape E (Fig. 2) are long measurements, each representing the sum of three shorter ones. Bookstein et al. (1985) advised elimination of such redundant variables. Mandibular shape B, mandibular length C, and mandibular length G (Fig. 2) are rooted on often-broken landmarks, thus, yielding many (≥ 10) missing values. The 10 remaining craniometric measurements, as well as dry mass of eye lens, form the basis of the following results, for a total of 144 muskrats collected in nine local populations (Table 1).

Mass of eye lens is not commensurable with the 10 craniometric measures. To overcome this scale problem, we analyzed natural logarithms instead of the values themselves. This transformation also linearizes eventual allometric relationships (Bookstein et al., 1985; Jolicoeur, 1963). By doing so, what is being analyzed is a set of multiplicative, rather than additive, relationships (Flury and Riedwyl, 1988). As a consequence, the assumed distribution of residuals is log-normal instead of normal, an assumption that could not be tested with the data at hand, but that is commonly accepted in morphometric studies.

The nine local populations showed significant multivariate differences for the 10 craniometric measures and mass of eye lens taken together (MANOVA Wilks' statistic $= 0.23$, $F = 2.67$, *d.f.* = 80,808, $P < 0.001$). Univariate differences were significant for all variables except interorbital constriction and length of mandibular toothrow. In the absence of any sex-related difference among population zones (Appendix I), we decided to pool sexes in further analyses. This had the advantage of increasing samples for statistical analysis. Mass of eye lens showed significant differences among zones ($F = 4.77$, *d.f.* = 8, 135, $P < 0.001$). Hence age, and possibly size, differences could account for at least part of the observed differentiation. Examination and removal of these confounding effects are explained in Appendix I, and illustrated in Fig. 3.

Global differentiation among local populations was maintained after the 10 craniometric variables were adjusted to constant mass of eye lenses (age-adjusted data, Fig. 3; testing for differences among zones, MANOVA Wilks' statistic $= 0.23$, $F = 2.66$, *d.f.* = 80,808, $P < 0.001$). A further decomposition of the age-adjusted data revealed that a small fraction only of the differentiation was related to an age-adjusted size component (Fig. 3; PC1 of the age-adjusted data accounted for 34% of the variation; Appendix I, factorial approach; testing for differences among zones, $F = 2.1$, *d.f.* = 8, 135, $P = 0.04$), while most of the differentiation was related to age-adjusted components of shape (Fig. 3; PC2–PC10 of age-adjusted data accounted for 66% of the variation; testing for differences among zones, MANOVA Wilks' statistic $= 0.25$, $F = 2.76$, *d.f.* = 72, 780, $P < 0.001$). Population zones thus were clearly differentiated in relation to shape of skull. Of the eight craniometric variables showing significant univariate differences among local populations, six still did so after age and size effects were removed. A canonical discriminant analysis of the age-and-size-adjusted data (Fig. 3) showed that length of braincase, width of braincase, zygomatic breadth, mandible height D, length of mandibular toothrow, and length of maxillary toothrow best discriminated shapes of skulls among these populations of muskrats.

The pairwise morphometric differences required to study the spatial pattern of differentiation, quantified as squared Mahalanobis distances among seven (zones M and Z excluded) means of local populations based on the 11 original ($\log_e$-transformed) morphometric variables (D^2 values; Table 4), were split into additive components homologous to the age and size adjustments (Fig. 3). They were calculated from mass of eye lens (age-related D^2), and from the 10 craniometric variables adjusted to constant mass of eye lens (age-adjusted D^2; Table 5, above the diagonal). Squared Mahalanobis distances were further calculated from the first principal component of the

TABLE 4.—*Morphometric differences, measured as squared pairwise Mahalanobis distances (D^2) among population zones; zones M and Z excluded (see text);* n = *100 individuals. Above the diagonal: unadjusted distances (based on 11 variables); below the diagonal: size-adjusted, shape-related distances (based on 10 principal components).*

Zone	C	E	J	L	N	O	T
C		4.946	5.272	4.036	7.998	12.960*	7.969*
E	4.580		9.345*	5.973*	12.020*	16.484*	6.245*
J	5.031	9.333*		3.637	5.090	15.132*	7.673*
L	2.993	5.803*	3.353		4.631	7.404*	2.307
N	7.986	11.779*	4.951	3.806		7.129	7.044
O	12.076*	16.378*	14.930*	7.398*	6.452		7.322*
T	6.260*	5.755*	7.001*	2.226	5.617	7.188*	

* Mahalanobis distances significantly different from zero at the Bonferroni-corrected 0.05/21 = 0.0024 probability level. Geographic zones are shown in Fig. 1.

age-adjusted data (age-adjusted, size-related D^2), and finally from the 2–10 age-adjusted principal components (age-adjusted, shape-related D^2, Table 5). Some of these squared-Mahalanobis-distance matrices are not reported here because they can be calculated from the data presented in Tables 4 and 5. The age-related D^2 is simply the difference between total (unadjusted, Table 4, above diagonal) and age-adjusted values (Table 5, below diagonal). In the same way, the age-adjusted, size-related D^2 between these zones is found by subtracting the age-adjusted, shape-related D^2 (Table 5, below diagonal) from the age-adjusted D^2 (Table 5, above diagonal). After Bonferroni correction of significance (Miller, 1977), giving a corrected significance level α^1 = 0.05/21 comparisons = 0.0024, two age-related distances, two age-adjusted, size-related, and 11 age-adjusted, shape-related distances were significantly positive. Some population zones thus differed for age or size, but most differences were related to shape.

The factorial decomposition into a general size component and subsequent size-adjusted shape components (Fig. 3; Appendix I; Mahalanobis distances based on the latter components are reported in Table 4, below diagonal) yielded a similar pattern of differentiation, related to shape but not to size. Results of this analysis are not reported in detail here.

Morphometric differentiation and spatial distances.—Mahalanobis distances among these seven zones, based on the unadjusted, age-adjusted, and age-adjusted, shape-related

TABLE 5.—*Morphometric differences, measured as squared pairwise Mahalanobis distances (D^2) among population zones; zones M and Z excluded (see text);* n = *100 individuals. Above the diagonal: age-adjusted distances (based upon 10 age-free variables); below the diagonal: age-adjusted, shape-related distances (based upon nine principal components).*

Zone	C	E	J	L	N	O	T
C		4.692	5.139	3.105	7.947	12.271*	6.401*
E	4.490		9.321*	5.760*	11.485*	16.378*	5.683
J	5.108	9.254*		3.269	4.748	14.915*	6.875*
L	3.084	5.674*	3.269		3.208	7.388*	2.226
N	6.335	10.818*	3.565	1.954		6.012	4.849
O	12.090*	16.378*	14.853*	7.312*	5.299		7.145*
T	6.066*	5.669*	6.718*	2.045	4.368	7.124*	

* Mahalanobis distances significantly different from zero at the Bonferroni-corrected 0.05/21 = 0.0024 probability level. Geographic zones are shown in Fig. 1.

TABLE 6.—*Spatial and morphometric distance matrices among population zones: Mantel statistics (associated probabilities in parentheses); zones M and Z excluded. Probabilities are based on 1,000 random permutations, except # based on 10,000.*

Morphometric → distance matrices	Unadjusted	Age-adjusted	Lens mass	Age-adjusted size-related	Age-adjusted shape-related
Straight	0.40	0.41	−0.12	−0.25	0.42
line	(#0.027*)	(#0.026*)	(0.339)	(0.100)	(#0.022*)
Along	0.34	0.34	−0.01	0.05	0.32
waterways	(#0.129)	(#0.145)	(0.455)	(0.276)	(#0.164)
Swimming	0.43	0.43	−0.02	0.09	0.40
(main river)	(#0.052)	(#0.051)	(0.462)	(0.335)	(#0.066)
Walking	−0.08	−0.08	0.01	−0.05	−0.07
(affluents)	(0.413)	(0.392)	(0.486)	(0.479)	(0.402)
Decision	0.64	0.65	−0.10	0.05	0.62
	(#0.005*)	(#0.006*)	(0.366)	(0.432)	(#0.006*)

* Significant at the 0.05 probability level.

datasets, are positively correlated with spatial distances measured either as straight lines or by the number of decisions (Table 6). These spatial distances are not related to the mass of eye lens, which is the age indicator. After decomposition, these relationships hold only for the component of shape (i.e., the shape-related datasets), but not for the age-adjusted component of size. They do not hold for distances measured along the waterways.

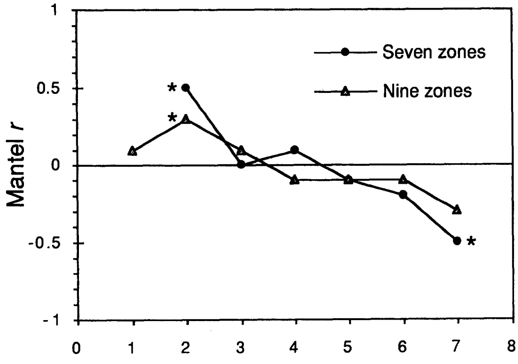

FIG. 5.—Mantel correlogram for the morphometric distances based on the age-adjusted dataset (triangles, nine zones; circles, seven zones after excluding M and Z): abscissa, decision distance classes, the single distance of value 8 (Table 1) is lumped with distance class 7; ordinate, standardized Mantel statistic correlating morphometric distances to the spatial weight matrix based on the corresponding distance class. Asterisks indicate significant values of the Mantel statistic ($P \leq 0.05$).

Mahalanobis morphometric distances among local populations are strongly correlated with decision distances, more so than with any other way of measuring distances (Table 6). The structure of this relationship, as revealed by a multivariate-Mantel-correlogram analysis involving the age-adjusted distance matrix and the decision distances for the seven population zones (Fig. 5) has two characteristics; first, at least one Mantel test is significant at the Bonferroni-corrected level $\alpha' = 0.05/\text{six}$ simultaneous tests = 0.008. This is the correction recommended by Oden (1984) for assessing correlograms. It confirms the presence of a significant spatial structure with respect to decision distances; second, autocorrelation is positive in the short distance classes, and negative in the large classes. This is characteristic of a spatial gradient (Legendre and Fortin, 1989; Sokal, 1979) and shows that cranial shape follows a spatial gradient along the course of the River, relative to decision distances among local populations. A similarly obtained correlogram computed from straight-line distances displayed no significant value.

DISCUSSION

Sex, age, and size.—In these analyses, sexes were pooled because sexual dimorphism was clearly not significant (Appendix I). Although some authors found differences in cranial measurements between male and female muskrats, they reported them to be weak and to concern mostly the general factor of size (Ruprecht, 1974; Sather, 1956). For example, Pankakoski and Nurmi (1986) found that in muskrats from Finland, the skulls of males averaged 1.5% larger than those of females. Other authors found no differences (Pietsch, 1970). The appropriateness of pooling sexes is corroborated by the lack of heterogeneity of sex ratios among local populations in the present study. It is hard to imagine how sexual dimorphism per se could induce a morphometric differentiation among local populations, in the absence of heterogeneous sex ratios.

Our analyses revealed size differences among local populations, part of which may be attributable to age. Thus, it appeared necessary to control this source of variation. The usefulness of adjusting craniometric variables to constant age to remove allometric influences of aging has been recognized by Pankakoski and Nurmi (1986) in their study of morphological differentiation among populations of muskrats in Finland.

We distinguished components related respectively to age, size, and shape. These labels may be unduly emphatic. It was implicitly assumed that mass of eye lens is only related to age, not to age-adjusted size. Data in support of this hypothesis are weak in general (Pucek and Lowe, 1975) and absent for muskrats. It also was assumed that the first principal component (of either the unadjusted, or the age-adjusted data) is only related to size, and the subsequent ones only to shape (Appendix I). Indeed, the first principal component of the age-adjusted data had the profile of a size axis, with all loadings positive and about equal (except for length of maxillary toothrow and length of mandibular toothrow, see Appendix I). The second and third principal components were left unchanged after applying the shearing technique (Bookstein et al., 1985), so they could be considered reasonably free from size-related influence. Most importantly, all components that we labelled age-related or size-related, were unrelated to the gradient of morphometric differentiation observed in this study.

The three datasets involving variation in shape of skull (unadjusted, age-adjusted, and age-adjusted, shape-related datasets) yield a similar spatial pattern of variation related primarily to decision distances among local populations, whereas the age structure, represented by the mass of eye lens, as well as the age-adjusted, size-related distances, varied independently from this pattern. As shown in Appendix I, killing of muskrats by landowners possibly affected the age structure of the local populations. Landowners were asked to refrain from trapping muskrats during the course of this study, but all of them did not comply with this request. Hence, while the three datasets describing shape of skull follow a similar spatial pattern of variation, the set of craniometric measures adjusted to constant mass of eye lens (age-adjusted distances) probably represents best the characteristics of the skull; it involves the least distortion of the data, while eliminating the potentially confounding effects of age.

Model of isolation by distance along corridors.—In the only other study dealing with morphometric differentiation of closely neighboring local populations of muskrats, Pankakoski and Nurmi (1986) reported a rather small degree of differentiation (mainly in size) among subpopulations of muskrats in Finland separated from one another by 1–10 km. In the present study, populations separated by 1.5–7 km present clearly differentiated shapes of skull. We have shown that the morphometric differentiation based on the age-adjusted component is not random, but structured as a recognizable spatial pattern, as demonstrated by Mantel tests and correlogram analysis. The same holds true for the unadjusted and the age-adjusted, shape-related distanc-

TABLE 7.—*Spatial and morphometric distance matrices among population zones: Mantel statistics (associated probabilities in parentheses); zones M and Z excluded. Above the diagonal, simple Mantel tests; below, partial Mantel tests. For partial Mantel tests, the matrix controlled for is stated on left of result. Probabilities are based on 1,000 random permutations, except # based on 10,000.*

Distance matrices		Straight line	Along waterways	Swimming (main river)	Walking (affluents)	Decision	Age-adjusted
Straight			0.46	0.54	−0.01	0.60	0.41
line			(0.029*)	(0.018*)	(0.490)	(0.003*)	(#0.026*)
Along	Age-adjusted→	0.38		0.93	0.53	0.89	0.34
waterways		(0.053)		(0.002*)	(0.029*)	(0.001*)	(#0.145)
Swimming	Age-adjusted→	0.45			0.18	0.92	0.43
(main river)		(0.025*)			(0.285)	(0.002*)	(#0.051)
Walking	Age-adjusted→	0.03				0.27	−0.08
(affluents)		(0.447)				(0.156)	(0.392)
Decision	Age-adjusted→	0.49					0.65
		(#0.018*)					(#0.006*)
Age-adjusted	Decision→	0.03			Straight line→	0.55	
		(#0.455)				(#0.010*)	

* Significant at the 0.05 probability level.

es. This pattern is more closely related to the decision distances than to the straight-line distances among population zones (Tables 6 and 7). Further evidence is the fact that all 10 predictions derived from the model of isolation by distance along corridors, in terms of values of the Mantel and partial-Mantel statistics, are supported by these data (Table 8).

The correlogram in Fig. 5 has the gradient shape predicted by the isolation-by-distance model, except for the expected return to negligible correlations at distances larger than the dispersal capabilities of the species (Sokal and Wartenberg, 1983). This is because our study area is small and easily crossed in a matter of days by dispersing muskrats; thus, the expected return to negligible correlations is not observed.

TABLE 8.—*Checking the 10 predictions of the isolation-by-distance-along-corridors model;* A *= straight-line distances,* B *= decision distances, and* C *= morphometric (age-adjusted) distances.*

Predictions of model	Relations as observed in Tables 6–7
$AB \times BC \approx AC$	$[0.60 \times 0.65 = 0.39] \approx 0.41$
$\lvert AB \rvert \geq \lvert AC \rvert$	$0.60 > 0.41$
$\lvert BC \rvert \geq \lvert AC \rvert$	$0.64 > 0.41$
$AB \neq 0$	$AB = 0.60$*
$BC \neq 0$	$BC = 0.65$*
$AB{\cdot}C \neq 0$	$AB{\cdot}C = 0.49$*
$BC{\cdot}A \neq 0$	$BC{\cdot}A = 0.55$*
$AC{\cdot}B = 0$	$AC{\cdot}B = 0.03$ ns
$\lvert AB{\cdot}C \rvert \leq \lvert AB \rvert$	$0.49 < 0.60$
$\lvert BC{\cdot}A \rvert \leq \lvert BC \rvert$	$0.55 < 0.65$

* Significant at the 0.05 probability level; ns, non-significant.

We have excluded zones M and Z from the spatial analysis on the grounds of higher spatial connectedness between these zones, and of M with the upper part of zone C. A correlogram based on all nine zones (triangles in Fig. 5) displays a gradient in shape similar to the one calculated for seven zones (circles in Fig. 5), but the Mantel statistics for the extreme classes of decision distance are not as large. Also, it may be argued that, if zone M may be entered by muskrats crossing straight from upper zone C, then the reverse possibility also exists. A correlogram based on six zones, excluding zone C, has exactly the same shape as with seven zones, with even higher correlations observed for the extreme distance classes (class 2, $r = 0.53$; class 7, $r = -0.55$), but lesser significance due to the smaller number of data values when only six zones are retained. The spatial gradient of morphometry in relation to decision distances thus remains apparent. This is in spite of the paucity of data when only six zones are retained, and despite distortions from the assumptions of the model when all nine zones are retained.

Considering our hypothesis of isolation by distance along corridors, the rather small amount of morphometric differentiation found by Pankakoski and Nurmi (1986) among subpopulations in Lake Lohja in Finland may be accounted for by a higher degree of connectedness among population zones in their area. Patton and Feder (1981) noticed that high connectedness may increase within-population heterogeneity. This may have allowed the muskrats to intermingle more freely, or at least spatially more at random in Lake Lohja, than in the La Houille River area, where barriers induced the formation of a morphometric cline along the course of the River.

If the morphometric gradient were more closely related to dispersal distance along waterways than to straight-line distance, the observed morphometric differentiation of local populations of muskrats would be expected to be strongly correlated with total distance along the waterways. It is interesting that we only observe a weak correlation. This may be due to the opposite effects of its two components, namely walking and swimming distances. Indeed, the correlation of morphometric differentiation with walking distances is insignificant ($r = -0.08$; Table 6), whereas it is positive with swimming distances ($r = 0.43$). This suggests that the barriers to dispersal of muskrats are not of a physical nature, as are the obstacles encountered by a dispersing muskrat along affluent tributaries (walking distances), but are rather of a social nature, e.g., related to territoriality (Chesser, 1983) of the animals installed along the main course of the River. But the latter speculation is based on weak evidence. The existence of a morphometric gradient as implied by our model of isolation by distance along corridors seems well founded. However, the nature of the barriers provoking this isolation, the intensity of which is related to the decision distances, remains to be confirmed. Future studies should disentangle the respective influences of social and environmental barriers on gene exchange, especially in view of the ever-increasing fragmentation of habitats left available to our fauna under the pressure of human activities (Bauchau and Le Boulengé, 1991).

The present study has contributed two important results. We have shown local morphometric variation to occur at a much finer spatial scale than was known for muskrats, thus confirming the existence of local patterns of differentiation even in this highly mobile mammal. Further, a microgeographic gradient also was found in the skull morphometry of local populations, running along the waterways that are preferential dispersal routes for this rodent. This gradient is measurable in terms of the number of decisions that muskrats have to make at confluences of rivers when moving from one population zone to another. Spatially restricted gene exchange is a parsimonious candidate mechanism to explain this variation.

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APPENDIX I

Sex as a potential confounding factor.—Males and females did not display significant differences in the 10 craniometric measurements, taken either together (MANOVA Wilks' statistic = 0.90, $F = 1.54$, *d.f.* = 10, 132, $P = 0.13$) or separately (only length of braincase approached significance, $F = 3.79$, *d.f.* = 1, 141, $P = 0.054$). The comparison still failed to reveal sexual dimorphism after controlling for age (craniometric values adjusted to constant lens mass) and for zone effects (zone average subtracted from each age-adjusted value); MANOVA test was not significant, only length of braincase was significantly different, $F = 5.10$, *d.f.* = 1, 141, $P = 0.03$. Finally, in a comparison limited to the subsample of adult individuals (15 males and 10 females with an estimated age >8 months), the MANOVA test also remained non-significant (Wilks' statistic = 0.63, $F = 0.82$, *d.f.* = 10, 14, $P = 0.6$), while only length of maxillary toothrow showed a significant univariate difference ($F = 7.69$, *d.f.* = 1, 23, $P = 0.01$), the average for males being only 4% larger than for females. The sex ratio (Table 1) also can be considered homogeneous across zones ($\chi^2 = 4.5$, *d.f.* = 8, $P = 0.8$). So, there is no indication of any sex-induced morphometric variability.

Age and size as potential confounding factors.—Age composition of the populations under study at least partly depended upon the skill in destroying muskrats by pond owners. Any influence of age upon morphometry could thus result in an artificial differentiation. Contrary to gender, age usually is not known, but must be estimated on the basis of some measured variable(s). It is not a categorical variable, and should be treated as a covariate. In mammals, age exerts a strong and usually nonlinear influence on the size of individuals; moreover, its influence on phenotypic traits may be complicated by allometric growth of certain parts of the body (Jolicoeur, 1963). Thus, it is important to separate components of size and shape when studying sources of differentiation among local populations. This complex problem has motivated a large amount of research (e.g., Bookstein et al., 1985; Flury and Riedwyl, 1988; Somers, 1989; Thorpe, 1983).

A simple way to eliminate spurious differentiation induced by the age structure would be to restrict the analyses to adults, but this would have drastically reduced the size of our sample. Generally, populations of muskrats consist of ca. 20% of adults in autumn (Errington, 1963; Le Boulengé and Le Boulengé-Nguyen, 1981), and this was true in our sample. A less-wasteful way consists of removing the age-induced variation in size. All craniometric measurements except length of maxillary toothrow, interorbital constriction, and length of mandibular toothrow, were positively correlated with mass of eye lens. The latter variable is an excellent indicator of age in muskrats (Le Boulengé, 1977; Vincent and Quéré, 1972), as well as in many other rodents (Pucek and Lowe, 1975). Its growth may depend on external factors (e.g., environment during youth—Le Boulengé, 1977; season of birth—Adamczewska-Andrzejewska, 1973), but this dependence is much smaller than for other morphometric traits (Pucek and Lowe, 1975).

Two possible approaches are available to express and remove age-related variation. Both are based on allometric-growth equations; taking logarithms of the morphometric variables then allows use of linear models (Bookstein et al., 1985; Jolicoeur, 1963; Thorpe, 1983). One assumes either 1) that some characteristic(s) entirely reflect(s) age-related variation, as is suggested for mass of eye lens, or 2) that no particular characteristic has such a privileged quality. Under the first assumption, removing the effect of age amounts to adjusting the morphometric variables to a constant value of the privileged indicator of age (regression approach). In the second case, it must be assumed that age affects general size; a combination of the morphometric variables representing general size must be constructed, and the morphometric variables must be adjusted to a constant value of this general size component (factorial approach; e.g., Bookstein et al., 1985). The technical steps of data manipulation are outlined below and shown in Fig. 3.

1. Regression approach.—The 10 $\log_e$-transformed craniometric variables were regressed against mass of eye lens ($\log_e$) within each local population, with the constraint that the slope parameter be the same across populations. Using these regressions, all craniometric variables were adjusted to constant mass of eye lens, taken as the overall mean calculated on the $\log_e$-transformed values for the 144 individuals. This corresponded to an age of 193 days, according to the growth curve for this region given in Le Boulengé (1977). All relationships were reasonably linear, and all craniometric variables were positively correlated with mass of eye lens (seven of them significantly so). The constraint of a common slope was imposed despite significant (but slight) heterogeneity of slopes for five variables, to avoid arbitrary differences among zones in the adjusted values depending on the value chosen as constant mass of eye lens (as usual in covariance analysis; e.g., Thorpe, 1983). Technically, the set of 10 craniometric variables adjusted to constant mass of eye lens, labelled "age-adjusted" in the text and in Fig. 3, was obtained by applying to the $\log_e$-transformed data the following linear transformation:

$$Y^*_{ijk} = Y_{ijk} - b_k(x_{ij} - \bar{x})$$
$$(k = 1, \ldots, 10 \text{ craniometric variables})$$

where Y_{ijk} is the value of the k-th $\log_e$-transformed craniometric variable and x_{ij} is $\log_e$-mass of eye lens for individual j from zone i; Y^*_{ijk} is that value adjusted to the mean $\log_e$-mass of eye lens $\bar{x}$; the slope b_k is the weighted average over zones, of slopes b_{ik} estimated by regression within zones.

2. Factorial approach.—A principal-component analysis (PCA) was applied to the pooled within-zone covariance matrix (Thorpe, 1983) of the 11 unadjusted (but $\log_e$-transformed) morphometric variables, including $\log_e$-mass of eye lens. The first principal component (PC1), associated with 66% of the within-zone variance, had the profile of a general-size-component axis, with all loadings positive (Jolicoeur, 1963), but presenting allometric relationships, as loadings were not all equal (Somers, 1989); mass of eye lens strongly dominated this axis, whereas lengths of mandibular toothrow and maxillary toothrow and interorbital constriction were almost unrelated to it. Size thus could have been removed by applying the above regression technique, using PC1 as the regressor; but due to the orthogonality of successive eigenvectors, this is tantamount to keeping the second and all subsequent PCs (Simar, 1980) as 10 size-adjusted components of shape.

A similar factorial decomposition using PCA was applied to the pooled within-zones covariance matrix of the age-adjusted craniometric data (Fig. 3). This yielded a variable (PC1, 34% of the within-zones variance of age-adjusted data) called age-adjusted component of size, that had the profile of a size axis, with all 10 variables positively and about equally loaded (except length of both toothrows, maxillary and mandibular, that were positively, but weakly, loaded); it also yielded a set of nine age-adjusted components of shape (PC2-PC10), with shape variations being related mainly to interorbital constriction, length of mandibular toothrow, and mandibular shape F. These age-adjusted components of shape also were expressed in terms of the original craniometric variables instead of component scores. Each age-adjusted craniometric variable was adjusted to a mean (i.e., zero) value of the age-adjusted component of size, PC1, following the above regression approach. This dataset was termed age-and-size-adjusted craniometric data (Fig. 3). It provided an easier base for appreciating the influence of the different craniometric measures in the differentiation among population zones, although for calculating the Mahalanobis distances, it was strictly equivalent to the age-adjusted components of shape.