Syst. Zool., 33(1):69–82, 1984

GEOGRAPHIC DISTANCE, ENVIRONMENTAL DIFFERENCE, AND DIVERGENCE BETWEEN ISOLATED POPULATIONS

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Abstract.—Evolutionary theory predicts that population divergence should be correlated with both geographic distance and environmental difference. This prediction was tested using 25 populations of *Goniobasis proxima* (Say), a snail restricted to small creeks in the piedmont and mountains of the southern Appalachians. Geographic distance was measured both over land and through water. Genetic difference was measured in three ways: an analysis of allozyme frequencies at seven polymorphic enzyme loci using gel electrophoresis, and two measures based on different variance components from 23 specially-screened morphological variables. Three measures of environmental difference were also calculated: one based on 11 water chemical variables; a second based on standardized water chemical variables plus four physical and biological variables; and a third based on diatom floral similarity. The correlations between all pairs of the resulting eight symmetric matrices were examined by calculating K_c statistics and tested using permutation. Population divergence was found highly correlated with geographic distance by all measures. Morphological divergence was also correlated with environmental difference. Isolation may, however, have precluded a correlation between divergence in allozyme frequencies and environmental difference. Thus, allozyme divergence among isolated populations seems more a function of gene flow restriction or time since isolation than selection. [Geographic variation; natural selection; gene flow; genic–environmental covariation; snails; gastropods.]

A great body of evolutionary theory predicts that genetic divergence between pairs of populations should be positively correlated with interpopulation distance and environmental difference (reviews by Felsenstein, 1976; Endler, 1977). The former relationship is expected due to an increasing time lag between the origin of novel mutations and their spread to populations separated by greater distances. The latter is an expected result of natural selection. These two relationships are probably often confounded, because environmental difference is likely to be correlated with geographic distance. But if the strength of the divergence/distance and divergence/environmental difference correlations could be separately compared, some light would be cast on the relative contributions of selection and gene flow restriction in promoting evolution.

Evidence of a relationship between geographic distance and genetic divergence has in fact been gathered in numerous studies, although methods of data analysis have varied widely (review by Gould and Johnston, 1972). One approach has been the examination of autocorrelations among allele frequencies at sites separated by varying distances through graphs (Sokal and Oden, 1978a, b; Jones et al., 1980). Patton and Yang (1977) and Guries and Ledig (1982) have calculated Pearson product-moment correlations between all entries in symmetrical matrices of genetic and geographic distances, although the independence assumption is violated. Jones et al. (1980) and Douglas and Endler (1982) have employed Mantel's (1967) *Z* statistics for similar purposes. Fewer investigators have examined the relationship between genetic divergence and overall environmental difference, perhaps due to the difficulty of measuring the environment. McKechnie et al. (1975) found a Pearson product-moment correlation between all entries in a matrix of Nei's genetic distances from butterflies and a matrix of en-

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vironmental differences. Douglas and Endler (1982) used Mantel tests to compare environmental with spot-polymorphism variation in guppies.

Recently, Dietz (1983) reviewed and evaluated the statistical methods that have been employed to estimate the association between pairs of distance matrices. She used permutation to test the significance of Mantel's Z, in light of Mielke's (1978) finding that Z is not asymptotically normal. She found that the power of the Mantel test varies greatly, depending on the specific distance measures used. The most consistently powerful test was one suggested by Hubert (1978) based on ranks and a component of Kendall's tau statistic, K_c.

The purpose of this study is to test evolutionary theory regarding the correlations between environmental difference, geographic distance, and population divergence. The study organism is *Goniobasis proxima* (Say), a freshwater snail particularly suitable to address these questions. Population divergence is estimated using two methods of combining a set of morphological measurements, along with allozyme frequencies at seven polymorphic loci. Environmental difference is also estimated in three ways: unstandardized water chemical data, standardized physical-chemical-biological data, and diatom floral data. Both land and water geographic distances are employed. I then test all pairs of the resulting eight matrices for association using K_c statistics.

METHODS

Study organism.—Goniobasis proxima is a member of the Pleuroceridae, a family of prosobranch (gill-breathing, operculated) freshwater snails found throughout North America. Pleurocerids are dioecious, and there is no evidence of asexual reproduction (Dazo, 1965). They require a year to mature and are capable of living several years (Stiven and Walton, 1967). The family has radiated in the rivers and streams of the southeastern United States to produce several endemic genera and around 500 nominal species (Tryon, 1873; Goodrich, 1940, 1942). Species identification depends heavily on rather unreliable shell characteristics, however, and pleurocerid taxonomy is currently in a confused state.

Goniobasis proxima is common in most small, softwater creeks in the piedmont and mountains on both sides of the Appalachian divide from Roanoke, Virginia, south into Georgia (Dillon, 1982). It is a convenient size (shell length 1–2 cm), is easily sampled year around, occurs in great numbers (sometimes hundreds per square meter), and lives well in the laboratory. Some information on population divergence in *Goniobasis* is already available (Chambers, 1978, 1980, 1982). But the most important advantage of using *G. proxima* for this study is its occurrence in numerous, highly discrete populations.

Goniobasis proxima population densities have the unusual tendency to increase as stream size decreases (Foin and Stiven, 1970; Foin, 1971). At any time, the great majority of the snails are crawling against the current, and this general upstream migration seems to more than compensate for the occasional individual that must loose its grip and wash downstream (Crutchfield, 1966; Krieger and Burbanck, 1976; Mancini, 1978). Eggs are cemented firmly onto rocks and hatch directly into crawling young that also move upstream. The ultimate reason for the upstream concentration of *G. proxima* is unknown. But the result is that the range of *G. proxima* is fragmented into a large number of isolated populations, separated from one another by mountains between drainages and by stretches of large, apparently uninhabited river. Thus, I was able to sample 25 discrete, natural populations from a small geographic area.

As might be expected, there is a great deal of divergence between *G. proxima* populations, in both details of shell morphology and allozyme frequencies. In a survey of 12 *Goniobasis* populations (six *G. proxima*) from southern Virginia and northwestern North Carolina, Dillon and Davis (1980) found that the average Nei's identity (Nei, 1972) between pairs of conspecific populations was unusually low

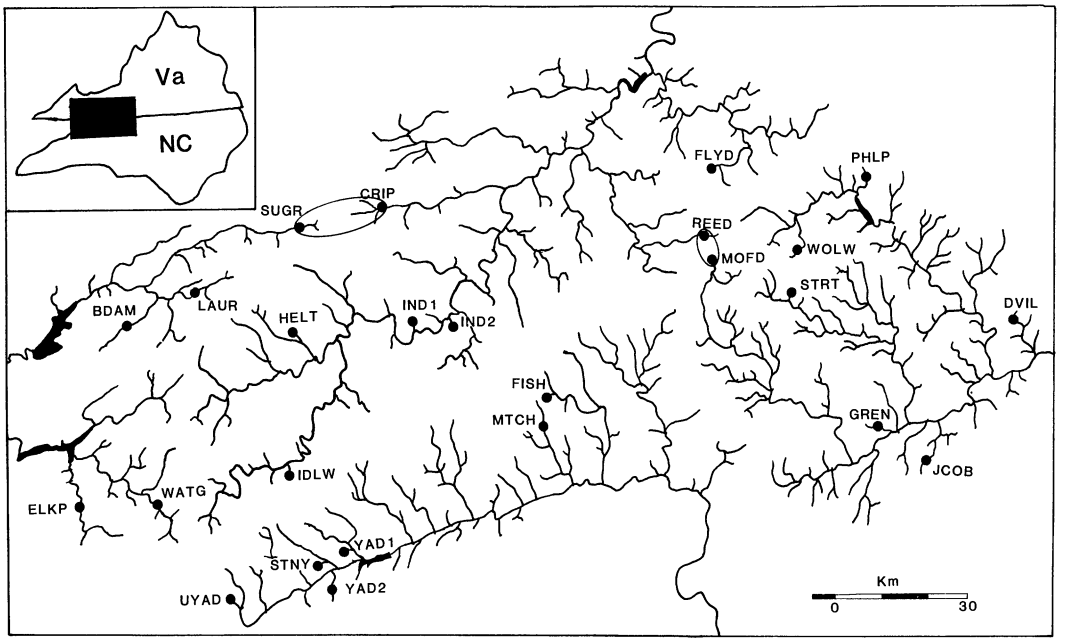

FIG. 1. *Goniobasis proxima* sample sites in Virginia and North Carolina. Locations of hypothesized Pleistocene stream capture encircled.

compared to values observed in other animals, as were average heterozygosities. Widely separated *G. proxima* populations often show fixation for alternative alleles at multiple enzyme loci. But in spite of this interpopulation divergence, *G. proxima* has allozyme frequencies very distinct from all other local *Goniobasis* species. Furthermore, no qualitative karyotypic or anatomical divergence has been observed in *G. proxima*, nor have any abrupt discontinuities in allele frequencies or external morphology been observed in the species range (Dillon, 1982). So although the widely separated *G. proxima* populations are quite diverse genetically, they at least constitute a readily recognizable unit below the genus level. Breeding experiments are ongoing.

Another benefit of using *G. proxima* is that environmental measurement of riverine habitats is comparatively easy. Unlike terrestrial or marine organisms, freshwater organisms are bathed in a medium that varies greatly in chemical composition, both temporally and spatially. There is considerable evidence that variation in ionic concentrations (particularly calcium, alkalinity, and pH) affects the distribution and abundance of many freshwater snails (review by Dillon and Benfield, 1982). The chemical constitution of water is easily measured, and thus there is an added dimension to environmental variation. Study of a river-dwelling organism is also advantageous because the correlation between distance and environmental difference may be assessed in two different ways. Populations may live on the same mountain slope in identical environments but be separated by hundreds of kilometers through water.

Study sites and interpopulation distances.—Figure 1 shows the northern third of *G. proxima*'s range and locates 25 populations sampled for this study. Each sample site was assigned a four-character name according to a local geographic feature. Precise locality data are given in Dillon (1982). Voucher specimens from all populations were deposited in the Academy of Natural Sciences of Philadelphia.

Sample populations were selected using several criteria. Most importantly, I chose areas to sample where the catchment was fairly undisturbed by man, without impoundments (even in the lowest reaches of the population) or major towns. Secondly, *G. proxima* needed to be sufficiently common for a large sample to be obtained. Third, I included two particular pairs of populations because of strong evidence of Pleistocene stream capture (Ross, 1969).

Goniobasis proxima populations may have ranges from 5 km to 50 km in length. Since the environment changes rapidly down a stream gradient, particularly in small streams, I used a standard method for choosing the precise site to sample each of the 25 populations. First, the downstream terminus of a population was defined as the collection station where at most two *G. proxima* were collected in five minutes. Then the upstream terminus was defined as the tip of the most distant permanent tributary inhabited by the snail. Often numerous tributaries were surveyed before the upstream terminus could be determined. The final sample sites shown on Figure 1 were estimated to be midway between the upstream and downstream termini for each population.

Water distance between populations was measured from downstream terminus to downstream terminus, not sample site to sample site. This was necessary to avoid biasing the estimate of relative migration by the geographic size of the snail population. Distances between downstream termini for every pair of populations connected through water was measured from U.S. Geological Survey 7.5-min topographic maps using a rolling map meter. A problem is that upstream, downstream, and across-stream distances are probably not equivalent with respect to migrating snails. However, it is not obvious as to how one should make an appropriate correction; thus, I have not taken into account this particular factor.

The 25 study populations occur in four separate river systems with freshwater connections either remote or nonexistent. Indeed, only 67 of the 300 pairs of populations could be realistically considered connected through water. The two stream captures believed to have occurred in this area are probably fairly recent and, as will be shown below, were apparently of little consequence to snail dispersal. Thus, a single dummy value higher than the highest water distance was entered in the 233 vacant positions in the water distance matrix. This should not have an adverse affect on the overall analysis, since my analyses are based on ranks and tested permutationally.

It seems clear that snail dispersal has occurred over land, perhaps in or on birds or semi-aquatic mammals. Aerial dispersal of mollusks is also a possibility and has been reviewed by Rees (1965). Thus, overland distances between all 300 pairs of populations were measured linearly, from sample site to sample site, on U.S. Geological Survey 1:250,000 topographic maps.

Environmental difference.—Standard methods (American Public Health Association et al., 1975) were used in the measurement of 11 water chemical variables. Standard method numbers are given where applicable: alkalinity (403, titration), calcium (306C, EDTA titrimetric), chloride (408A, argentometric), iron (310A, phenanthroline), magnesium (313C, calculation), nitrogen (ammonia, 418C, phenate), nitrogen (nitrate, phenoldisulfonic acid, similar to 419E), phosphorus (total orthophosphate, 425F, ascorbic acid), potassium (AA spectrophotometry), sodium (AA spectrophotometry), and sulfate (427C, turbidimetric). Each of these variables was measured four times, once a season from July 1980 to April 1981. Iron, potassium, and sodium were not determined in the summer.

For the "chemical environment" measurement, I assumed that all 11 water chemistry variables (measured in mg/liter) were important to snail biology in proportion to their standard deviations. For each season, simple Euclidean distance was calculated over 11 variables between all pairs of the 25 populations. When a measurement was missing, the mean of the other three seasons was sub-

stituted. Then the mean of the four seasonal Euclidean distances was used as the "chemical environment" estimate of environmental difference.

The same 11 variables were used in the "standardized environment" measure, but four new variables were added. Temperature was measured to the nearest 0.5°C from midstream at mid-depth each of the four seasons. Every season except winter, total water flow was estimated (m^3/sec) using cross-sectional area of the stream and current speed derived from floats. Flow rates were log transformed, due to some extreme values in large rivers and spring floods. Average stream gradient, an indirect measurement of current speed over the whole stream inhabited by the snails, was determined from topographic maps.

Several species of trematodes (Lang et al., 1970) and one protozoan were commonly found infecting *G. proxima* during dissection. The percent parasitism for each population (systematically overestimated by considering large snails only) was arcsine transformed and included as variable 15.

For the "standardized environment" measure, all 15 variables were given equal weight by standardization. Then, for each season, Euclidean distance in standard deviations was calculated over 15 variables between all pairs of the 25 populations. Means (or single values) were again substituted for missing values, and the mean of the four seasonal Euclidean distances was used as the estimate of standardized environmental difference.

Diatom floral difference was used as an independent estimate of the similarity of pairs of environments. Diatoms have been widely used for such purposes (see reviews by McIntire, 1975; Patrick, 1973), primarily because they are extremely diverse, grow and spread rapidly, and are believed to be capable of dispersing great distances airborne. (This minimizes biogeographic problems.) Collection of diatom samples may present a problem, however, because small variations in current and light may cause variation in the diatom flora over short distances. As a partial remedy for human bias, I let *G. proxima* itself be the diatom collector, and used the diatoms in snail guts as an indirect measurement of the environment the snails were experiencing.

Radioactive tracer studies (Malone and Nelson, 1969; Elwood and Nelson, 1972) suggest that diatoms are not accidentally ingested as *Goniobasis* grazes over the stream bed, but rather are actively sought as food. There is also evidence (Dillon and Reimer, unpubl. data) that *G. proxima* does not randomly ingest all diatoms present. But as long as snails from the 25 populations are fairly constant in their grazing methods, comparisons between populations should be valid.

During the July 1980 sample trip, five large *G. proxima* were collected from a range of habitat types at each of the 25 sample sites. Snails were placed immediately in bottles of 70% ethyl alcohol, which killed them tightly contracted in their shells and helped clean shell surfaces of attached periphyton. In the laboratory, shells were brushed and washed thoroughly, cracked with pliers, and the animal removed whole. The head-foot regions, with operculums and any contaminating diatoms, were excised. The remainder of the tissue samples from each sample of five snails, containing their stomachs and intestines, were combined, washed, and digested in boiling nitric acid. The residue after 30 min of digestion was composed almost entirely of clean diatom frustules.

A pilot study (detailed in Dillon, 1982) suggested that 1,000 diatom cells should be counted and identified for each of the 25 samples. The coefficient of Jaccard, S_j (Sneath and Sokal, 1973), was then calculated between pairs of sites based on the presence of given species at both locations, and $1 - S_j$ was used as a measure of diatom floral difference. The Jaccard coefficient does not use species abundances, but high intrasite variability in diatom abundance probably renders relative abundance data spurious; it also does not use negative matches.

Genetic difference.—In preliminary stud-

ies, Dillon and Davis (1980) and Dillon (1982) sampled representative *G. proxima* populations from the entire study area and screened them for variation of approximately 20 enzyme loci. Horizontal starch gel electrophoresis was performed using methods similar to those described by Ayala et al. (1973). Most of the buffers and histochemical staining procedures employed were based on recipes of Shaw and Prasad (1970) and Bush and Huettel (1972), using agar overlay for the stains where practical. Details of all electrophoretic procedures, buffers, and stains are given in Dillon and Davis (1980) and Dillon (1982).

The preliminary screenings uncovered polymorphism at seven enzyme loci, so only these seven were examined for the main study that is the subject of this report. Approximately 40 to 50 snails were sampled from each of the 25 populations. The products of each of the seven loci were compared using at least two buffer systems chosen to represent the range of pH values tolerated by the protein in question. Samples were frozen and comparisons were made with all previously detected variants of similar mobilities when a new variant was discovered. Buffers employed were tris-citrate pH 6 (TC 6), tris-EDTA-borate pH 8 (TEB 8), tris-EDTA-borate pH 9.1 (TEB 9), and Poulik's (1957) discontinuous buffer system, gel approximately pH 8.8 and vessel approximately pH 8.

Enzyme loci examined and buffer systems employed were as follows: (1) *Aph*, alkaline phosphatase, EC 3.1.3.1 (TEB 9, Poulik). A special staining procedure was used so that putatively, the product of only a single locus was apparent. (2) *Est*, arylesterase, EC 3.1.1.2 (TEB 8, TEB 9, Poulik). The slowest of several loci was the only one scored. (3) *Gpi*, glucosephosphate isomerase, EC 5.3.1.9 (TC 6, Poulik). (4) *Lap*, aminopeptidase (cytosol), EC 3.4.11.1 (TEB 9, Poulik). (5) *Mpi*, mannosephosphate isomerase, EC 5.3.1.8 (TC 6, TEB 8). (6) *Odh*, octopine dehydrogenase, EC 1.5.1.11 (TC 6, Poulik). (7) *Xdh*, xanthine dehydrogenase, EC 1.2.1.37 (TEB 8, Poulik).

Results at the seven loci were combined by calculating Rogers' (1972) genetic distances for all pairs of samples. Unlike the more commonly reported Nei's (1972) genetic distances, Rogers' distances obey the triangle inequality. It is also misleading to compute Nei's distances if monomorphic loci have been deliberately excluded.

Population divergence was also estimated using the overall morphological similarity of the snails. Problems with this approach fall into three categories: character variance that is very low, very high, or not genetic. As a partial remedy for the first two of these problems, I screened a large number of morphological variables by requiring that they vary substantially among three populations believed to be genetically different. These three populations (CRIP, PHLP, and YAD1) represented the three races of *G. proxima* described by Dillon and Davis (1980) based on allozyme criteria. It was also hoped that this screening would eliminate variables that, for miscellaneous nongenetic reasons, might vary significantly between other populations not genetically different.

Ten apparently healthy, unparasitized, sexually mature females were measured for each of the three races. A special protocol was followed to insure that the 10 snails selected were near the maximum size obtained in the population as a whole. After taking six shell measurements, the shell was carefully cracked with pliers and the living snail removed intact. First, the snail was placed in 70% ethyl alcohol buffered at pH 7 for exactly 5 min at room temperature to kill it and contract it to some standard degree. Then it was transferred to a Petri dish of water, it was dissected, and 27 body measurements and 6 counts were made using uniform techniques. Dillon (1982, 1984) provides detailed methods and diagrams for all measurements and counts taken.

A stepwise multivariate analysis of variance (BMDP7M; Jennrich and Sampson, 1981) was used to determine if any of the 33 measurements varied substantially among the three races. It was decided *a priori* that the "substantial" variance would be attributed to variables with F values corresponding to the 99% confidence level in at least one of the multiple steps of the

MANOVA. However, for several reasons, including heterogeneity of sample variances, the true significance of these F values is unknown.

Seven steps were required before all variables meeting the above criterion were included on the MANOVA. Of the 33 metric variables initially examined, 21 variables varied substantially among the three races. They were: shell height (last three whorls only), body whorl height, third whorl width, aperture width, rostrum length and width, tentacle length, width between eyes, operculum length, body length, digestive gland length, cerebral ganglion length and width, pleural ganglion width, pedal ganglion diameter, buccal mass length and width, radula length and width, jaw length, and outer marginal tooth length. These measurements were made on 10 snails from each of the remaining 22 populations.

Using Mann-Whitney U-tests and Kolmogorov-Smirnov tests, two of the six count variables, number of gill filaments and number of cusps on the outer marginal tooth, were found to vary significantly among the three races. These were also determined on the remaining 22 populations.

Although size variance generally has some genetic component, nongenetic factors such as age and nutrition surely make an important contribution. It has been suggested that size variance can be at least partially removed by disregarding the first component of a principal component analysis (Blackith and Reyment, 1971; Atchley et al., 1976). If growth is modeled as an increase in the size of the various body components proportional to their variance, the component analysis should be based on the covariance matrix. But if growth is better modeled by size increases evenly distributed without regard to absolute variance, the correlation matrix is a better choice for the principal component analysis. Because I had no evidence that either of these assumptions was more realistic, I used both techniques.

Thus two separate principal component analyses (BMDP4M; Frane and Jennrich, 1981) were performed, one on the correlation matrix of the 21 measurements based on 250 individuals, and a second on the covariance matrix. All measurements were recorded in millimeters. I disregarded variance on the first principal component, and any subsequent nonsignificant component. Factor scores for the 250 individuals on the remaining components were used for the calculation of Mahalanobis D^2 statistics between all pairs of the 25 populations (BMDP7M; Jennrich and Sampson, 1981). The two count variables, when square-root transformed to normality (Sokal and Rohlf, 1969:384), had variances comparable to those of the factor scores, and were also included in the calculations of the D^2 statistics. The matrix of D^2 values based on principal components from the correlation matrix was designated "morphology 1," and the similar matrix based on those from the covariance matrix was called "morphology 2."

The procedure just described—a MANOVA on the three races followed by a principal component analysis—is not a substitute for heritability studies. It was designed to screen out some sorts of nongenetic variance, not screen for genetic variance. That morphological variance passing this two-stepped test has a significant genetic component will be taken as an assumption.

Overall analysis.—Eight 25 × 25 symmetric matrices have been described thus far: three measures of genetic difference (allozyme, morphology 1, morphology 2); three measures of environmental difference (diatom, standardized environment, and chemical environment); and two measures of geographic distance (land and water). Thus 28 separate K_c tests comparing all pairs of the eight matrices were performed, using a Fortran program supplied by E. J. Dietz (1983). Values of P associated with each K_c statistic were estimated from 2,000 permutations.

RESULTS

Environment.—Raw data and sample statistics for the 15 environmental measurements and the diatom analysis are given in Dillon (1982), along with matrices of standardized and chemical Euclidean dis-

tances. Of the chemical variables, calcium, magnesium, and carbonate (alkalinity) had by far the largest means and variances. These three dominated the other variables in the chemical measure of environmental difference. Calcium, magnesium, and alkalinity, along with total hardness, pH, and conductivity, are all generally intercorrelated in natural fresh waters (e.g., Sepkoski and Rex, 1974), and all vary widely according to surface geology. The entire study area, with the exception of a northern slice that includes SUGR and CRIP, is underlain by crystalline rock such as gneiss and schist. Thus, calcium concentrations in the southern 23 sites were comparatively low, ranging from 20 mg/liter at BDAM in the fall to 2 mg/liter in the fall of STRT. By contrast, the SUGR site had a mean calcium concentration of 32 mg/liter and the CRIP site had a mean of 44 mg/liter. The effects of unusually high hardness on shell morphology in the CRIP population of *G. proxima* were explored by Dillon and Davis (1980).

Two other major trends were apparent in the physical and chemical data. One was the tendency for the piedmont streams of the east, particularly DVIL, JCOB, and GREN, to have warmer summer temperatures and cooler winter temperatures due to greater surface water contributions, along with lower stream gradients and generally higher ionic concentrations. The second was the tendency of *G. proxima* populations in the Holston/Watauga drainages of the west (WATG, ELKP, LAUR, and SUGR) to extend their ranges into large streams.

Over 150 diatom species were identified in the 25 samples, comprising 23 genera. Small pennate diatoms seem to constitute the bulk of the diet of *G. proxima*. By far the most common species, at least 15% in all sites, was *Achnanthes deflexa* Reimer. It and the second most common species, *Achnanthes minutissima* Kutz., comprised an average of 74% of the diatom cells identified in each sample. Other species occasionally common were *Achnanthes lanceolata* (Breb.) Grun., *Gomphonema parvulum* (Kutz.) Kutz., *Fragilaria vaucheriae* (Kutz.) Peters., and *Navicula decussis* Østr. More complete discussion of these results will be published elsewhere (Dillon and Reimer, unpubl. data).

Electrophoresis.—Table 1 gives allozyme frequencies encoded by seven presumptive loci for the 25 *G. proxima* populations. In Table 1, isozyme classes are identified by the mobilities in gels of a standard pH, and reported in millimeters plus or minus an arbitrarily set constant of 100. If a class of isozymes had identical mobilities in the standard gel but were distinct in gels of a second buffer system, the suffixes "S," "F," and "VF" (slow, fast, and very fast) were appended.

Of the 36 isozyme classes resolved, seven would have been missed had only one buffer system been used. (This includes two isozyme classes that were resolved in the standard gel conditions upon which Table 1 was based, but not in a second set of conditions.) Six of these hidden isozymes were at the highly variable *Odh* locus, with 16 alleles total. This supports the observation that loci known to be highly polymorphic become proportionately more polymorphic when screened using additional gel conditions (Coyne and Felton, 1978; Coyne et al., 1979). Techniques for discovering "hidden variation" do not tend to be successful when the locus examined seems to have low polymorphism beforehand.

The amount of divergence seen in populations of *G. proxima* is extremely high. A complete matrix of Rogers' genetic distances is given in Dillon (1982). The most different pair of populations was LAUR and PHLP, apparently sharing no alleles at six of the seven loci examined. Divergence of this magnitude is highly unusual on conspecific populations. Without the knowledge that a full range of intergrades exists between LAUR and PHLP, their conspecific status would be questionable.

Morphology.—The first principal component based on the covariance matrix accounted for 90% of the total variance. Principal components 2 through 10 were also found to be significant in the covariance analysis, together accounting for

another 10% of the variance. As might be expected, the variables most important in these nine components were those with the largest means (for example the four shell measurements, overall body length, and digestive gland length; Dillon, 1984). The remaining 11 components, with just 0.2% of the variance, were disregarded.

Size accounted for a much smaller portion of the variance when modeled using the correlation matrix; the first principal component accounted for a relatively modest 34% of the total. The eigenvalues of the next five components were also greater than 1.0, together accounting for another 39% of the variance. Generally the characters with the smallest means were most important in principal components 2 through 6, for example, measurements from the head and central nervous system (Dillon, 1984). The last 15 components, accounting for 27% of the variance combined, were disregarded.

Mahalanobis D^2 statistics were calculated between all pairs of populations, using 7 variables (5 components + 2 counts) for morphology 1 (correlation) and 11 variables for morphology 2 (covariance). Complete matrices are given in Dillon (1982). Generally, the morphological distances were quite high. Although statistical inference on values of D^2 is precluded by variance heterogeneity, values of F were calculated for each D^2 as a qualitative aid to understanding similarities. Considering all 300 entries in each 25 × 25 symmetric matrix, there were only four values of F below the 0.95 level in the morphology 1 matrix and three in the morphology 2 matrix. These generally identified pairs of populations that were close geographically or had similar allozyme frequencies, such as GREN/JCOB, GREN/MTCH, IDLW/IND2, and STNY/YAD2.

Overall analysis.—Table 2 shows that all but 10 of the 28 possible pairs of the eight data matrices are significantly correlated. With the exception of diatom floral difference, which is correlated with none of the other matrices, almost every entry in Table 2 reflects a high probability of positive association. The three measures of population divergence are strongly intercorrelated, as are the two geographic distance matrices and the two physico-chemical environmental measures. No matter how population divergence was measured or how geographic distance was measured, divergence was highly correlated with distance. Interestingly, although both chemical and standardized environmental difference are correlated with morphology 1 and morphology 2, neither is correlated with population divergence estimated using allozymes.

DISCUSSION

It seems clear from the geology and geography of the study region that no gene flow now occurs among most of these populations. The broad outlines of the present Appalachians and their drainages were probably formed by the collision of the continents in the Paleozoic, long before the first *Goniobasis* fossils occur in the Cretaceous (Henderson, 1935). With few exceptions, present topography seems to be the result of erosion proceeding at different rates in rocks of varying resistance, combined with general upwarping over the entire area (Hack, 1969, 1979). In the softer, sedimentary layers of rock found west of the Blue Ridge and north of the study area, the drainage systems have adjusted to differences in bedrock by multiple piracies. However, in resistant crystalline rock, such as that which underlies all but the northernmost tip of *G. proxima*'s range, piracy has been rare. Only 67 of the 300 pairs of populations are now connected by water, and with very minor exceptions, local drainage relationships have remained unchanged since the appearance of *Goniobasis*.

There is some evidence that gene flow between even closely neighboring populations has been very low for thousands of generations, in spite of Pleistocene climatic fluctuations. First consider the six pairs of populations that are relatively close through water. The BDAM/LAUR pair is very similar genetically (Rogers distance = 0.088). But the other pair of populations neighboring closely on the

TABLE 1. Allozyme frequencies at seven enzyme loci for 25 *G. proxima* populations.

Locus and allele	DVIL	PHLP	WOLW	JCOB	STRT	GREN	MOFD	REED	FLYD	IND1	IND2	IDLW	HELT
Odh 101											0.04		
103												0.04	
105						0.01							
105.5													
106											0.39	0.50	
107				0.62		0.01	0.07						
109S												0.43	
109F			0.01	0.38	1.00	0.97				1.00	0.57	0.01	1.00
110													
111													
111.5													
112			0.99				0.93	1.00	1.00				
113S	0.27												
113F	0.57	0.99										0.02	
113VF													
115	0.14	0.01				0.01							
n	35	39	50	45	38	67	51	69	45	100	79	70	54
Lap 98		1.00	1.00										
96	1.00			1.00	1.00		1.00	1.00	1.00				
94						1.00				0.96	0.68	1.00	1.00
92										0.04	0.32		
88													
n	40	37	44	13	36	31	43	69	46	100	48	39	50
Gpi 105							0.01						
102			0.90	1.00	1.00	1.00	0.99	1.00	0.86	0.98		1.00	1.00
100	1.00	1.00	0.10						0.14	0.02			
99											1.00		
98													
97													
n	41	39	47	39	37	59	45	66	46	100	73	66	54
Est 106	0.06					0.30							0.15
103			0.04	1.00	1.00	0.70	0.07			0.63	1.00	0.91	0.85
100	0.94	1.00	0.96				0.93	1.00	1.00	0.37		0.09	
n	34	39	50	18	35	32	51	63	45	55	67	65	51
Xdh 98	1.00			1.00	1.00	1.00		0.30			1.00	1.00	1.00
96		1.00	1.00				1.00	0.70	1.00	1.00			
n	43	39	50	39	18	53	48	62	45	100	76	67	51
Mpi 100													
95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
n	41	39	49	47	35	61	45	66	46	100	73	66	51
Aph 104	0.20	1.00	1.00	0.11	0.36	0.41	1.00	1.00	1.00	0.11	0.63	0.79	0.38
100	0.80			0.89	0.64	0.59				0.89	0.37	0.21	0.62
n	10	37	47	9	35	11	28	37	45	53	66	71	51

same side of the river, STNY/YAD1, has a genetic distance of 0.294. This is despite the fact that environmentally they are the most similar pair of sites in this study. And the four pairs of close neighbors on opposite sides of large rivers have genetic distances ranging from 0.228 to 0.510. Compare these values to the two pairs of stream capture populations absolutely isolated since the Pleistocene (Ross, 1969). The MOFD/REED pair has a genetic distance of 0.069 and the SUGR/CRIP pair

TABLE 1. Continued.

Locus and allele	CRIP	SUGR	LAUR	BDAM	ELKP	WATG	UYAD	STNY	YAD2	YAD1	MTCH	FISH
Odh 101												
103												
105									0.01			
105.5											0.55	1.00
106									0.27	0.56		
107												
109S												
109F	1.00	1.00	0.14	0.76	1.00	0.91	0.20			0.18	0.45	
110									0.39			
111									0.32	0.02		
111.5			0.86	0.24								
112												
113S												
113F						0.09	0.78	1.00		0.24		
113VF							0.02					
115												
n	100	39	55	42	52	49	53	50	42	100	49	26
Lap 98												
96								0.55	1.00	1.00	0.31	1.00
94			1.00	1.00	1.00	1.00	1.00	0.45			0.69	
92												
88	1.00	1.00										
n	100	20	64	43	36	43	37	48	38	100	39	17
Gpi 105									0.02			
102	1.00	1.00	1.00	1.00	0.20	0.19	0.75	0.99	0.90	1.00	0.98	0.69
100												
99												
98					0.80	0.81	0.25		0.07			
97								0.01	0.01		0.02	0.31
n	100	33	55	51	57	50	53	50	47	100	50	26
Est 106									0.28			
103	1.00	0.90	1.00	1.00	0.65	0.59	0.38	1.00	0.52	0.69	1.00	0.94
100		0.10			0.35	0.41	0.62		0.20	0.31		0.06
n	30	15	54	51	51	50	53	50	48	40	50	26
Xdh 98			1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
96	1.00	1.00										
n	100	39	55	51	51	50	53	49	46	100	50	26
Mpi 100		0.17					0.07		0.08	0.18		
95	1.00	0.83	1.00	1.00	1.00	1.00	0.93	1.00	0.92	0.82	1.00	1.00
n	100	41	49	51	51	50	49	50	41	100	50	26
Aph 104					0.47	0.53	0.53	0.50	0.21	0.92	0.29	0.98
100	1.00	1.00	1.00	1.00	0.53	0.47	0.47	0.50	0.79	0.08	0.71	0.02
n	30	17	49	51	48	49	47	37	46	13	50	26

has a genetic distance of 0.039. Neither of these pairs lives in particularly similar environments. Overland dispersal between MOFD/REED and SUGR/CRIP, given their separation, seems unlikely. There is little evidence of dispersal between other populations that are close over land but not water (FISH/MTCH = 0.311, STRT/WOLW = 0.670). These observations combine to suggest that migration between even neighboring populations is currently negligible to absent.

TABLE 2. Values of P for K_c statistics calculated between pairs of eight symmetric matrices.

	Population divergence			Geographic distance		Environmental difference	
	Allozyme	Morphology 1	Morphology 2	Land	Water	Standard	Chemical
Morphology 1	0.003						
Morphology 2	0.004	0.001					
Land distance	0.001	0.001	0.002				
Water distance	0.033	0.005	0.007	0.001			
Standard environmental	0.208	0.010	0.012	0.063	0.005		
Chemical environmental	0.087	0.001	0.001	0.102	0.045	0.004	
Diatoms	0.218	0.118	0.284	0.446	0.245	0.129	0.065

The long-term stability of drainages in the study area suggests that either the environment, or the dispersal capabilities of the snail, or both, may have been very different in the Tertiary from what is observable today. And indeed, the allele frequencies shown in Table 1 suggest that rates of gene flow have been first relatively high and then relatively low for long periods of time. Nearly all alleles fall into two classes: those that are present in all four drainages (Holston and Watauga considered together); and those that are localized in only a single population or a few neighboring populations. In all 36 alleles recognized, none can be identified with a particular drainage. It would seem that all alleles either arose during a period when relatively rapid spreading even across drainages was possible, or during a subsequent period when dispersal was very difficult.

Divergence between populations of *G. proxima* is strongly correlated with geographic distance, regardless of how divergence or distance is measured. Apparently this relationship can exist with negligible gene flow. Perhaps the greater the population distance, the longer the time since a common ancestor was shared, and the greater the opportunity for divergence due to accumulation of new mutations and selection. On the other hand, the rarity of gene flow may have precluded any correlation between Rogers' genetic distance and environmental difference. As mentioned earlier, although some alleles are widespread, many others are quite restricted. It may be that the lack of gene flow has prevented the spread of beneficial alleles and cline formation.

Significant gene flow is apparently unnecessary for a correlation between environmental difference and morphological divergence, however. It is certainly possible that in spite of the precautions taken, *G. proxima*'s morphological variation may still have a significant nongenetic component that is tracking environmental variation. But the body shape of a snail is likely to be under complex, polygenic control, and there are probably many different ways to arrive at an overall shape that is best suited for a particular environment. Isolated populations inhabiting similar environments may evolve similar morphologies independently.

In summary, both selection and gene flow restriction (or more appropriately in most cases, time since isolation) seem quite important for promoting morphological divergence in isolated populations. There is no evidence that either evolutionary agent is more important. However, if divergence is measured in highly isolated populations using allozymes, rate of gene flow or time since isolation seem more important than selection for promoting evolution.

ACKNOWLEDGMENTS

This work is dedicated to my wife, Shary, who did as much of the dirty work as I did, and understood. Also furnishing indispensable advice and support was my advisor, George M. Davis. E. Jacquelin Dietz provided the computer program for the overall analysis. Major contributions of time and intellect were made

by S. M. Chambers, S. Friant, J. Gillespie, R. Horwitz, C. W. Reimer, R. E. Ricklefs, T. Uzzell, and my father, R. T. Dillon, Sr. Also helpful were R. Burton, A. Davis, A. E. Dunham, J. T. Hack, R. Hershler, P. Kiry, R. Mahoney, R. R. Sokal, R. Thomas, R. C. Vrijenhoek, and my wife's family, the Bryans. Room and board on my field trips were provided by my grandparents, Mr. and Mrs. O. D. Williams and Mrs. A. G. Dillon, and my aunt and uncle, Mr. and Mrs. V. Hubbard.

The University of Pennsylvania Department of Biology and the College of Charleston furnished computer time. The electrophoretic analyses were performed in the Laboratory of Molecular Genetics, Department of Malacology, Academy of Natural Sciences of Philadelphia. This work is based on a dissertation submitted to the University of Pennsylvania in partial fulfillment of the requirements for the Ph.D. degree. It was primarily supported by an NSF grant for improving doctoral dissertation research, No. DEB-8023344.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. Fourteenth edition. Am. Public Health Assoc., Washington, D.C.

ATCHLEY, W. R., C. T. GASKINS, AND D. ANDERSON. 1976. Statistical properties of ratios. I. Empirical results. Syst. Zool., 25:137–148.

AYALA, F. J., D. HEDGECOCK, G. S. ZUMWALT, AND J. W. VALENTINE. 1973. Genetic variation of *Tridacna maxima*, an ecological analogue of some unsuccessful evolutionary lineages. Evolution, 27:177–191.

BLACKITH, R. E., AND R. A. REYMENT. 1971. Multivariate morphometrics. Academic Press, New York.

BUSH, G. L., AND R. N. HUETTEL. 1972. Starch gel electrophoresis of tephritid proteins. A manual of techniques. International Biological Programme. Working group on fruit flies. Project phase I report. 56 pp.

CHAMBERS, S. M. 1978. An electrophoretically detected sibling species of "*Goniobasis floridensis*" (Mesogastropoda: Pleuroceridae). Malacologia, 17:157–162.

CHAMBERS, S. M. 1980. Genetic divergence between populations of *Goniobasis* occupying different drainage systems. Malacologia, 20:63–81.

CHAMBERS, S. M. 1982. Chromosomal evidence for parallel evolution of shell sculpture pattern in *Goniobasis*. Evolution, 36:113–120.

COYNE, J. A., W. F. EANES, J. A. M. RAMSHAW, AND R. K. KOEHN. 1979. Electrophoretic heterogeneity of α-glycerophosphate dehydrogenase among many species of *Drosophila*. Syst. Zool., 28:164–175.

COYNE, J. A., AND A. A. FELTON. 1978. Genic heterogeneity of two alcohol dehydrogenase loci in *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics, 87:285–304.

CRUTCHFIELD, P. J. 1966. Positive rheotaxis in *Goniobasis proxima*. Nautilus, 79:80–86.

DAZO, B. C. 1965. The morphology and natural history of *Pleurocera acuta* and *Goniobasis livescens* (Gastropoda: Cerithiacea: Pleuroceridae). Malacologia, 3:1–80.

DIETZ, E. J. 1983. Permutation tests for association between two distance matrices. Syst. Zool., 32:21–26.

DILLON, R. T., JR. 1982. The correlates of divergence in isolated populations of the freshwater snail, *Goniobasis proxima* (Say). Ph.D. dissertation, Univ. Pennsylvania, Philadelphia.

DILLON, R. T., JR. 1984. What shall I measure on my snails? Allozyme data and multivariate analysis used to reduce the non-genetic component of morphological variance in *Goniobasis proxima*. Malacologia (in press).

DILLON, R. T., JR., AND E. F. BENFIELD. 1982. The distribution of pulmonate snails in the New River of Virginia and North Carolina, U.S.A.: Interaction between alkalinity and stream drainage area. Freshwater Biol., 12:179–186.

DILLON, R. T., JR., AND G. M. DAVIS. 1980. The *Goniobasis* of southern Virginia and northwestern North Carolina: Genetic and shell morphometric relationships. Malacologia, 20:83–98.

DOUGLAS, M. E., AND J. A. ENDLER. 1982. Quantitative matrix comparisons in ecological and evolutionary investigations. J. Theor. Biol., 99:777–795.

ELWOOD, J. W., AND D. J. NELSON. 1972. Periphyton production and grazing rates in a stream measured with a ^{32}P balance method. Oikos, 23:295–303.

ENDLER, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, New Jersey.

FELSENSTEIN, J. 1976. The theoretical population genetics of variable selection and migration. Annu. Rev. Genet., 10:253–280.

FOIN, T. C. 1971. The distribution pattern of the freshwater prosobranch gastropod *Oxytrema proxima* (Say). J. Elisha Mitchell Sci. Soc., 87:1–10.

FOIN, T. C., AND A. E. STIVEN. 1970. The relationship of environment size and population parameters in *Oxytrema proxima* (Say) (Gastropoda: Pleuroceridae). Oecologia (Berl.),.5:74–84.

FRANE, J., AND R. JENNRICH. 1981. Factor analysis. Pages 656–684 *in* BMDP. Biomedical computer programs (W. J. Dixon, ed.). Univ. California Press, Berkeley.

GOODRICH, C. 1940. The Pleuroceridae of the Ohio River system. Occas. Pap. Mus. Zool. Univ. Michigan No. 417.

GOODRICH, C. 1942. The Pleuroceridae of the Atlantic coastal plain. Occas. Pap. Mus. Zool. Univ. Michigan No. 456.

GOULD, S. J., AND R. F. JOHNSTON. 1972. Geographic variation. Annu. Rev. Ecol. Syst., 3:457–498.

GURIES, R. P., AND F. T. LEDIG. 1982. Genetic diversity and population structure in pitch pine (*Pinus rigida* Mill.). Evolution, 36:387–402.

HACK, J. T. 1969. The area, its geology: Cenozoic development of the southern Appalachians. Pages 1–17 *in* The distributional history of the biota of the southern Appalachians. Part I. Invertebrates (P.

C. Holt, ed.). Virginia Tech Res. Div. Monogr. 1, Blacksburg.

HACK, J. T. 1979. Rock control and tectonism—Their importance in shaping the Appalachian highlands. U.S. Geol. Surv., Professional Pap. 1126-B.

HENDERSON, J. 1935. Fossil non-marine mollusca of North America. Geol. Soc. Am., Spec. Pap. No. 3.

HUBERT, L. J. 1978. Generalized proximity function comparisons. Br. J. Math. Statist. Psychol., 31:179-192.

JENNRICH, R., AND P. SAMPSON. 1981. Stepwise discriminant analysis. Pages 711-740 *in* BMDP. Biomedical computer programs (W. J. Dixon, ed.). Univ. California Press, Berkeley.

JONES, J. S., R. K. SELANDER, AND G. D. SCHNELL. 1980. Patterns of morphological and molecular polymorphism in the land snail *Cepaea nemoralis*. Biol. J. Linn. Soc., 14:359-387.

KRIEGER, K. A., AND W. D. BURBANCK. 1976. Distribution and dispersal mechanisms of *Oxytrema* (=*Goniobasis*) *suturalis* Haldeman (Gastropoda: Pleuroceridae) in the Yellow River, Georgia, U.S.A. Am. Midl. Nat., 95:49-63.

LANG, B. Z., L. N. GLEASON, AND F. W. RACHFORD. 1970. Effects of *Metagonimoides oregonensis* Price, 1931 (Trematoda: Heterophyidae) and *Mosesia chordeilesia* McMullen, 1936 (Trematoda: Lecithodendriidae) on *Goniobasis proxima* (Say) (Gastropoda: Pleuroceridae) in North Carolina. J. Parasitol., 56: 836-838.

MALONE, C. R., AND D. J. NELSON. 1969. Feeding rates of freshwater snails (*Goniobasis clavaeformis*) determined with cobalt^{60}. Ecology, 50:728-730.

MANCINI, E. R. 1978. The biology of *Goniobasis semicarinata* (Say) (Gastropoda: Pleuroceridae) in the Mosquito Creek drainage system, southern Indiana. Ph.D. dissertation, Univ. Louisville, Louisville, Kentucky.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27:209-220.

MCINTIRE, C. D. 1975. Periphyton assemblages in laboratory streams. Pages 403-430 *in* River ecology (B. A. Whitton, ed.). Univ. California Press, Berkeley.

MCKECHNIE, S. W., P. R. EHRLICH, AND R. R. WHITE. 1975. Population genetics of *Euphydryas* butterflies. I. Genetic variation and the neutrality hypothesis. Genetics, 81:571-594.

MIELKE, P. W. 1978. Clarification and appropriate inferences for Mantel and Valand's nonparametric multivariate analysis technique. Biometrics, 34:277-282.

NEI, M. 1972. Genetic distance between populations. Am. Nat., 106:283-292.

PATRICK, R. 1973. Use of algae, especially diatoms, in the assessment of water quality. Pages 76-95 *in* Biological methods for the assessment of water quality. American Society for Testing and Materials, Philadelphia, ASTM STP 528.

PATTON, J. L., AND S. Y. YANG. 1977. Genetic variation in *Thomomys bottae* pocket gophers: Macrogeographic patterns. Evolution, 31:697-720.

POULIK, M. D. 1957. Starch gel electrophoresis in a discontinuous system of buffers. Nature, 180:1477-1479.

REES, W. J. 1965. The aerial dispersal of mollusca. Proc. Malacol. Soc. Lond., 36:269-282.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Publ., 7213:145-153.

ROSS, R. D. 1969. Drainage evolution and fish distribution problems in the southern Appalachians of Virginia. Pages 277-292 *in* The distributional history of the biota of the southern Appalachians. Volume 1 (P. C. Holt, ed.). Virginia Tech Res. Div. Monogr. 1, Blacksburg.

SEPKOSKI, J. J., AND M. A. REX. 1974. The distribution of freshwater mussels: Coastal rivers as biogeographic islands. Syst. Zool., 23:165-188.

SHAW, C. R., AND R. PRASAD. 1970. Starch gel electrophoresis of enzymes—A compilation of recipes. Biochem. Genet., 4:297-320.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. Freeman, San Francisco.

SOKAL, R. R., AND N. ODEN. 1978a. Spatial autocorrelation in biology. 1. Methodology. Biol. J. Linn. Soc., 10:199-228.

SOKAL, R. R., AND N. ODEN. 1978b. Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. Biol. J. Linn. Soc., 10:229-249.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. Freeman, San Francisco.

STIVEN, A. E., AND C. R. WALTON. 1967. Age and shell growth in the freshwater snail, *Goniobasis proxima* (Say). Am. Midl. Nat., 78:207-214.

TRYON, G. W., JR. 1873. Land and freshwater shells of North America. Part IV, Strepomatidae. Smithson. Misc. Collect. No. 245.

Received 16 February 1983; accepted 17 December 1983.