MALACOLOGIA, 1984, 25(2): 503–511

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*

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ABSTRACT

The pleurocerid snail *Goniobasis proxima* (Say) inhabits small streams in the piedmont and mountains of the southern Appalachians. The purpose of this study was to identify the morphological variables most useful in estimating genetic divergence between isolated populations of this snail. I made 33 measurements on ten large, mature females from each of three races of *G. proxima* using standardized techniques. These variables were screened by requiring that they vary substantially among the three races. Multivariate analysis of variance showed that 12 of the 33 measurements did not meet this requirement, including 7 of the 9 foot and body measurements. These 12 variables were generally eliminated because they had very low variances both within and between populations, although one might expect measurements on such elastic structures as the gill and osphradium to vary excessively.

The remaining 21 measurements were then made on ten individuals from each of 22 additional *G. proxima* populations. Principal component analyses were performed on both the correlation and covariance matrices of the 21 measurement variables calculated over all 250 individual snails, pooling within and between population variance. The first principal component, representing size variance, was disregarded, and the 21 measurements were ranked by their contributions to the variance on the significant principal components remaining. Among the 21 variables, there was a strong inverse correlation between variance and coefficient of variation. Not surprisingly, the variables with large means and variances were most important in the non-size, significant principal components from the covariance matrix. However, the variables with small means and variances were most important in the correlation matrix analysis. Variables of any size and from any part of the anatomy were found potentially useful, but it is recommended that all measurements be taken on structures of comparable variance. Work presented elsewhere suggests that measures of overall population divergence based on morphological variance as treated in this study are correlated with interpopulation geographic distance and environmental difference.

Key words: morphometrics; genetics; electrophoresis; divergence; mollusks; *Goniobasis*.

INTRODUCTION

Since the nineteenth century, a great deal of study has been devoted to morphometrics, and a wealth of knowledge has accumulated (reviews by Blackith & Reyment, 1971; Oxnard, 1978). One principal objective of morphometric analysis has been biological classification (reviews by Jardine & Sibson, 1971; Sneath & Sokal, 1973). However, systematists most often prefer to erect classifications based on discrete, particularly binary, characters if at all possible. The analysis of metric variables (morphometrics in the strict sense) is generally applied as a "last resort" for several reasons. First, the collection and analysis of metric data can be time-consuming, often requiring a computer and considerable statistical expertise. Second, it can be difficult to identify measurements that vary significantly. Given the number of individuals to be measured, it may be found that some metric traits do not vary at all, while others may vary excessively within groups. And finally, non-genetic variance doubtless contributes substantially to most if not all metric variation.

Non-genetic variance in metric characters

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can come from many sources. Some of the most obvious sources are extrinsic, as for example, oysters growing to fit their attachment site or soft water eroding a shell apex. Some intrinsic but not additively genetic sources of morphological variation, such as sex, reproductive condition, and health may also make significant contributions. Overall size variation is a particular problem. Although size variation surely has some genetic component, a large fraction of the variation observed in wild populations is typically due to age and nutrition. Finally, a great deal of measurement error is to be expected. In mollusks, for example, the size of the soft parts may be primarily a function of contraction due to preservation method or expansion due to the placement of a dissecting pin.

My research has recently focused on populations of *Goniobasis proxima* (Say), a pleurocerid snail living in small, isolated softwater creeks of the Appalachian Mountains and piedmont from Virginia to Georgia. In a larger study, I examined the correlations between population divergence, environmental difference, and geographic distance in order to estimate the relative importance of selection and gene flow restriction in the evolution of *G. proxima* (Dillon, 1982, 1984). Because the 25 *G. proxima* populations under study did not differ qualitatively in shell, anatomy, or karyotype, I estimated population divergence using protein electrophoresis and morphometrics. The purpose of this paper is to report the results of a screening of 33 measurement variables, in which I analytically remedy some of the problems with the application of morphometrics outlined above.

METHODS

The analysis was composed of two stages. In the first stage, I screened the morphological variables by requiring that they vary appreciably among three populations believed to be genetically different. These three populations represented the three races of *G. proxima* described by Dillon & Davis (1980) based on allozyme criteria. (They share no alleles at a minimum of two enzyme loci.) Variables failing this test fall into one of three categories. Some may simply be invariant in all *G. proxima* populations, due perhaps to developmental constraints and/or strong selection. Other measures may be so variable that values are not appreciably different between any pair of populations. This could be the result of extreme measuring error, for example. A third group of variables failing the screening procedure are those that may, in fact, vary substantially among some *G. proxima* populations other than the three selected for this test. There is certainly some chance that such variables do have a large genetic component and are thus useful for estimating genetic divergence. But because they do not vary appreciably among populations believed to be genetically different, it would also seem possible that any large differences among populations subsequently examined are environmentally induced or are the product of chance alone.

Approximately 100 individuals were collected from each of three populations representing the different races of *G. proxima*. Race A was represented by snails collected from station *Yad1*, on Naked Creek in the upper Yadkin drainage, race B was represented by station *Crip* from Cripple Creek of the New River drainage, and race C was represented by station *Phlp* from Nicholas Creek, a tributary of the Dan River. Complete locality data for these sites are given in Dillon (1982). The snails were held alive in aerated tanks at 15°C and fed commercial fish food until dissection.

From each population, 10 snails were selected using a procedure designed to eliminate some intrinsic non-genetic variance. First the largest individual in the tank was chosen. (Since these snails were collected by hand, this would be nearly the maximum size for the population.) Measurements were made on the shell, the shell was carefully cracked with pliers, and the living animal removed intact. Males, obviously parasitized individuals, or those showing reduced or discolored digestive gland were discarded and the next largest individual selected. This procedure was repeated until 10 large, healthy, sexually mature females were obtained.

Snails meeting the above criteria were placed in 70% ethyl alcohol buffered at pH 7 for exactly 5 minutes at room temperature. Afterward they were transferred to a Petri dish of water for dissection. All details of the dissection, including individual orientation and pin placement, were kept uniform. A total of 33 measurements was made on each of the 30 individuals (various methods of Davis & Carney, 1973). These variables were selected to cover a broad range of anatomical characters and with an eye toward repeatabil-

ity of measurement. They are listed in Table 1 and shown diagrammatically in Fig. 1.

The six shell measurements were made using vernier calipers. The remaining 27 measurements were made using an ocular micrometer at magnifications ranging from 12× to 100×. Length measurements were the maximum dimension of the particular organ under examination, and width measurements were generally the maximum dimension perpendicular to the length. Shell width and third whorl width were the maximum distance across the whorls (see Fig. 1), even though this was not perpendicular to shell length. Pedal ganglion diameter was the maximum dimension, generally 11 o'clock to 5 o'clock when the head of the animal was oriented towards 6 o'clock. Ganglia were fixed with Bouins solution before measurement. The jaw and radula were isolated by dissolving the entire buccal mass in commercial bleach (0.5% sodium hypochlorite).

TABLE 1. Mean and standard deviation in millimeters for measurements made on three races of *Goniobasis proxima*.

Variable number	Race A		Race B		Race C	
	Mean	SD	Mean	SD	Mean	SD
Shell						
1. Shell height (3 whorls)	14.85	.96	13.37	1.10	12.06	.58
2. Body whorl height	10.96	.55	10.51	.86	9.51	.41
3. Shell width	6.41	.33	6.73	.65	6.02	.27
4. Third whorl width	3.89	.37	3.08	.29	2.67	.29
5. Aperture length	6.38	.41	6.51	.84	5.56	.39
6. Aperture width	3.35	.22	3.88	.47	3.33	.21
External head						
7. Rostrum length	1.314	.096	1.147	.157	1.365	.136
8. Rostrum width	1.955	.137	2.182	.122	2.006	.139
9. Tentacle length	1.306	.138	1.126	.167	1.091	.117
10. Width between eyes	3.003	.159	3.144	.261	2.862	.198
11. Operculum length	4.305	.256	3.890	.503	3.546	.277
12. Operculum width	2.784	.190	2.706	.438	2.412	.333
Body						
13. Body length	23.69	2.39	22.36	3.97	18.84	2.01
14. Digestive gland length	13.23	2.63	12.40	3.07	9.70	1.13
15. Egg groove length	2.221	.332	2.381	.666	1.666	.256
16. Egg groove width	.704	.184	.651	.106	.623	.074
17. Pallial oviduct length	5.375	.466	8.218	4.01	3.954	.470
18. Pallial oviduct width	1.189	.235	1.052	.330	.755	.121
19. Gill length	5.724	.398	5.650	1.03	5.153	.629
20. Osphradium length	2.096	.224	1.846	.347	1.849	.171
21. Osphradium width	.186	.056	.178	.074	2.13	.072
Central nervous system						
22. Cerebral ganglion length	.627	.057	.721	.038	.666	.067
23. Cerebral ganglion width	.390	.043	.386	.051	.406	.040
24. Pleural ganglion length	.447	.065	.502	.070	.441	.085
25. Pleural ganglion width	.269	.021	.261	.039	.231	.022
26. Pedal ganglion diameter	.361	.028	.421	.059	.412	.056
Trophic apparatus						
27. Buccal mass length	1.963	.216	2.166	.184	2.260	.125
28. Buccal mass width	1.779	.114	2.064	.135	2.018	.118
29. Radula length	3.503	.313	4.133	.181	4.098	.247
30. Radula width	.384	.014	.417	.033	.394	.015
31. Jaw length	1.172	.065	1.270	.117	1.211	.104
32. Jaw width	.619	.021	.662	.085	.615	.056
33. 2nd. marginal tooth length	.209	.007	.244	.008	.239	.006

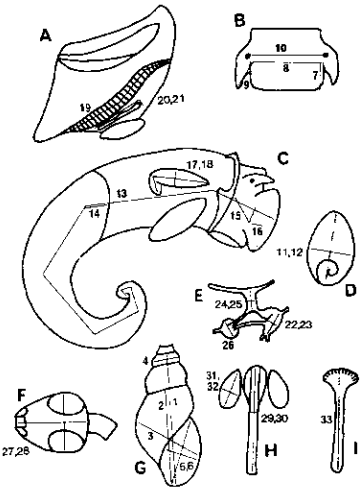

FIG. 1. Schematic diagrams showing 33 measurements made for initial screening of *G. proxima* morphological variables. See Table 1 for explanation of variable numbers. A, ventral surface of excised mantle. B, dorsal aspect of head. C, snail removed from shell. D, operculum. E, left side of central nervous sytem. F, dorsal aspect of buccal mass. G, shell. H, radular ribbon. I, second marginal tooth.

Jaws were dried flat on the slide before measurement.

A stepwise multivariate analysis of variance, BMDP7M (Jennrich & Sampson, 1981), was employed to determine if any of the metric variables varied substantially among the three races. The stepwise method was necessary because the number of variables was greater than the number of observations. At step 1, the variable selected was the one with the highest F value from an analysis of variance of the three groups. Then F values were recalculated for each of the remaining variables as in a two variable ANOVA where the variable selected in step 1 was included. At step 2, the variable with the highest F value was again selected, and F values were recalculated as in a three variable ANOVA where the variables in steps 1 and 2 were included. This process was repeated until no remaining variable had an F value corresponding to the 99% confidence level. Variables already included in the analysis of variance were removed if their F values dropped below acceptable levels during the stepping process. Only those variables with F values corresponding to the 99% confidence level at least once during the multiple stepping process were included in the second stage of the screening process.

The F value corresponding to the 99% confidence level was arbitrarily selected as a restrictive criterion. However, proper statistical inference cannot be made on these F values. Statistical inference from a multivariate analysis of variance assumes variance homogeneity. But in the 33 measurements × 3 groups = 99 separate variance estimates made for this analysis, values ranged over three orders of magnitude. Thus the true significance of these F values is unknown.

Variables passing the first stage test may still owe a great deal of their variance to simple size differences. Those variables showing the highest correlations with growth would seem most likely to be influenced by non-genetic factors such as age and nutrition. So the purpose of the second stage of this analysis was to rank the variables according to an estimate of the contribution of size.

Ten individuals from each of 22 additional populations were analyzed in the second stage (locality data in Dillon, 1982). Only a subset of the variables listed in Table 1 was examined. Otherwise the collection, dissection, and measurement techniques were identical to those used for the first three populations.

Principal component analysis has been suggested as a method of identifying some of the variance due to size, including correlated changes in shape (Blackith & Reyment, 1971; Atchley *et al.*, 1976). Principal components (PC's) can be extracted from either the covariance or correlation matrix of the measurements. If it is assumed that growth involves increase in all metric variables proportional to their coefficients of variation, analysis of the correlation matrix is appropriate. But if it is assumed that growth is best modelled as an increase in size proportional to the absolute variance of each measurement, analysis of the covariance matrix is more appropriate. Because I had no evidence that either of these assumptions was more realistic, I used both techniques to identify size-correlated variables.

Two separate principal component an-

alyses (BMDP4M, Frane & Jennrich, 1981) were performed, one on the covariance matrix of all measurements taken on the 250 individuals, the other on the correlation matrix. These were called the *covamorph* and *corrmorph* analyses, respectively. I disregarded variance on the first principal component, and tested the significance of the remaining PC's using one of two methods. For the *corrmorph* analysis, the simple rule-of-thumb method was employed that PC's with eigenvalues less than 1.0 should be disregarded. For the *covamorph* analysis, I used the method of Lawley (1956):

$$r(N-1)(\log \bar{\lambda} - \overline{\log \lambda}) = \chi^2,$$

$$\text{d.f.} = \frac{r(r+1)}{2} \quad 1$$

where N is the number of observations, r is the number of eigenvalues claimed to be equal under the null hypothesis (the smallest ones), and $\bar{\lambda}$ is the average of these last r eigenvalues. This is a test that there are no meaningful principal component directions corresponding to the last r eigenvalues. If m variables were measured, acceptance of the null hypothesis means that the first $m-r$ eigenvalues are the only ones significant.

The correlation or covariance of the original variable with a principal component is called the "loading" of that variable on that PC. Loadings adjusted by the size of the eigenvalues are measures of the contributions of particular variables to the variance represented by the PC. I used the sum of the loadings of each variable on the non-size, significant PC's as a ranking method. High-ranking variables (those with much variance uncorrelated with size) would be particularly recommended for future morphometric studies.

RESULTS

Population means and standard deviations for each of the 33 measurement variables taken on the three races are presented in Table 1. The F statistics for each variable at each of the seven steps required in the stepwise MANOVA are presented in Table 2. Twelve variables did not attain F values corresponding to the 99% confidence level at any step: shell width, aperture length, pallial oviduct length and width, gill length, operculum width, pleural ganglion length, egg groove length and width, osphradium length and width, and jaw width.

The results of the principal component analysis on the covariance matrix of the 21 remaining variables were somewhat surprising (Table 3). Principal component 1, the size component, accounted for 89.9% of the total variance, even though only the largest female snails were picked from each population in an effort to minimize variance due to size. The large eigenvalue is attributable both to high size variance and high covariances among the characters as they grow. The fact that 10 snails from each of 25 divergent populations in diverse habitats could be so similar in size relationships over a number of variables implies that measurement error was not a severe problem. Principal components 2 through 10 were found to be significant in the *covamorph* analysis, together accounting for 10.0% of the variance (Table 3). The remaining 11 components, with just 0.2% of the variance, were disregarded.

Size accounted for a much smaller portion of the variance when modelled using the correlation matrix; the first PC accounted for a relatively modest 34% of the total (Table 4). This implies that the variables with high absolute variances emphasized in the *covamorph* analysis have a higher proportion of "size" variation than do the low-variance measures. The eigenvalues of the next five PC's were also greater than 1.0, together accounting for another 39% of the variance. The last 15 principal components, accounting for 27% of the variance combined, were disregarded.

DISCUSSION

It might be expected that measurements taken on some of the hard structures, such as shell width, aperture length, operculum width, and jaw width, would not vary appreciably over three populations given a sample size of 10. However, a major result of the first stage screening was that, when properly controlled, measurements taken on even the most pliable structures can have remarkably low coefficients of variation. Examination of Tables 1 and 2 shows that, with only a few exceptions, the 12 variables were eliminated because of insufficient, not excessive, variation. It would seem advisable not to measure features of the body cavity and foot, as seven of the nine variables in that category were eliminated. But once again, even such elastic

TABLE 2. F values from stepwise multivariate analysis of variance of three *Goniobasis proxima* races based on 33 measurements. Variables above the diagonal were entered into the MANOVA. Refer to Table 1 for full names of variables.

	Step 0	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Degrees of freedom to enter:		2, 27	2, 26	2, 25	2, 24	2, 23	2, 22	2, 21	2, 20
Degrees of freedom to remove:		—	2, 27	2, 26	2, 25	2, 24	2, 23	2, 22	2, 21
Variables retained:								
33,MGLN	49.66	49.66	28.07	15.95	19.35	32.18	7.22	9.87
4,TWWD	26.93	13.29	13.29	18.76	17.19	5.16	4.80	4.42
31,JWLN	21.49	9.32	14.04	14.04	17.05	18.31	18.75	19.86
13,BDLN	10.95	6.98	10.53	13.21	13.21	15.52	18.36	18.22
1,SHHT	5.86	13.12	8.98	8.14	10.09	10.09	12.96	11.88
8,RSWD	8.94	6.83	9.91	8.13	7.50	10.08	10.08	13.19
7,RSLN	17.83	9.33	9.81	7.76	5.54	4.02	6.83	6.83
2,BWHT	1.40	10.28	7.75	5.06	5.63	.03	.46	.64
6,APWD	6.18	3.45	4.08	5.50	.60	2.40	1.29	3.45
9,TNLN	6.45	11.52	9.57	7.79	2.40	1.19	2.96	1.72
10,EYWD	1.99	7.36	2.05	1.07	.19	.12	.96	1.02
11,OPLN	3.43	7.11	2.11	1.13	5.11	1.18	.22	.79
14,DGLN	7.34	5.37	5.72	6.66	.13	.04	.05	.01
22,CGLN	11.58	2.50	2.62	4.01	1.06	.12	.10	.07
23,CGWD	6.85	5.37	5.03	3.81	.92	1.53	1.23	.91
25,PGWD	4.69	6.05	2.51	2.37	1.69	1.89	1.15	.98
26,PDIA	6.68	5.09	4.70	2.38	7.31	6.52	4.87	5.64
27,BMLN	6.11	.83	.67	.49	.22	.17	.90	.57
28,BMWD	14.87	.54	.58	.46	.75	3.84	1.15	1.30
29,RDLN	22.22	1.30	5.44	4.76	4.37	4.23	3.57	3.25
30,RDWD	15.83	4.63	6.32	2.24	1.24	.67	.58	.69
Variables eliminated:								
3,SHWD	1.25	1.63	1.09	1.06	.41	3.20	3.79	3.73
5,APLN	.26	2.31	.70	.75	.15	2.90	1.99	1.73
12,OPWD	.30	3.04	.98	.93	5.57	4.33	1.42	.72
15,EGLN	1.97	3.43	2.04	2.25	.01	.12	.05	.26
16,EGWD	1.95	2.92	2.67	2.42	3.75	3.65	2.45	4.19
17,POLN	3.91	.19	.50	1.32	.87	.63	.06	.06
18,POWD	.59	1.77	.61	.30	1.44	.34	.27	.51
19,GLLN	2.74	3.67	1.40	1.42	2.04	1.00	.83	.76
20,OSLN	3.09	.93	.09	.10	1.16	1.06	.25	.43
21,OSWD	.28	.26	.50	.50	.08	.11	.38	.48
24,PGLN	5.12	1.36	.96	.20	.66	.64	1.53	.42
32,JWWD	2.60	.18	1.25	2.44	2.18	3.48	1.89	1.79

and deformable organs as the osphradium and gill do not show excessive standard deviations.

The means of the 21 metric variables remaining covered a broad range of values, from 0.2 mm to 15 mm. As might be expected, the measurements with the largest means generally had the largest variances and loaded most heavily on the discarded first PC in the *covamorph* analysis. Nevertheless, these variables (e.g., shell, operculum, and body lengths) still covaried highly with the next nine PC's. The rankings computed for the 21 variables in Table 3 correspond closely to their means and absolute variances.

The results of the *corrmorph* analysis present a contrast, because the variables with the smallest means and absolute variances tended to have the largest coefficients of variation. Table 4 shows that, disregarding the first PC, the most important measurements from the correlation-based PCA were those taken on the smallest organs, such as the ganglia of the central nervous system and

TABLE 3. Results of principal component analysis on the covariance matrix of 21 *G. proxima* measurements, N = 250.

Variable name	Factor loadings										Rank
	1	2	3	4	5	6	7	8	9	10	
SHHT	.686	.766	.059	−.244	.021	.024	−.007	.036	−.013	−.062	1
BWHT	.359	.623	.027	.146	−.021	−.114	−.072	−.059	.000	.060	2
TWWD	.252	.138	.013	−.212	.033	.101	.068	−.031	.039	.100	5
APWD	.079	.270	.041	.261	−.110	.182	−.013	−.002	.013	−.010	3
DGLN	.025	−.207	.502	.026	.009	−.010	.006	−.003	.000	.000	4
BDLN	.035	−.100	−.371	.013	−.010	.006	−.010	.002	.000	.000	9
OPLN	.143	.189	−.006	.083	−.116	−.089	.157	.069	−.006	.015	6
RSLN	.026	.061	−.004	.046	.056	−.019	.006	.003	.070	−.045	12
RSWD	.043	.069	−.014	.066	.100	.021	.061	−.046	−.046	−.009	10
TNLN	.023	.036	−.011	.050	.035	−.057	.028	−.030	.112	−.020	11
EYWD	.085	.104	−.021	.107	.095	.004	.083	−.075	−.043	−.034	8
BMLN	.016	.050	−.012	.038	.037	.043	.017	.017	.054	−.011	13
BMWD	.035	.059	−.013	.057	.039	.031	.035	−.017	.002	−.002	14
CGLN	.008	.006	−.005	.013	−.009	.006	.004	−.003	.010	.001	16
CGWD	.005	.009	−.001	.004	.002	.003	.000	.001	.003	.000	21
PGWD	.003	.004	−.002	.004	−.002	.006	.002	−.004	.002	.002	19
PDIA	.006	.010	−.002	.002	−.007	.009	.005	.001	.004	.002	17
JWLN	.002	.045	.007	.053	.007	.027	−.001	.024	−.003	−.005	15
RDLN	.043	.107	−.003	.160	.178	.008	−.023	.108	−.008	.043	7
RDWD	.001	.011	−.003	.012	.002	−.001	.004	.000	.004	.001	18
MGLN	.000	.005	−.001	.010	.005	−.001	.001	.001	.002	.000	20
Eigenvalue—											
	19.240	1.195	.397	.256	.086	.073	.048	.032	.026	.023	
Cumulative variance—											
	.898	.954	.972	.984	.988	.991	.994	.995	.996	.998	

features of the head. Shell height and body whorl height, the most prominent variables in the *covamorph* analysis, ranked sixteenth and twenty-first in the *corrmorph* analysis. Conversely, the most important variable from the *corrmorph* analysis, pedal ganglion diameter and cerebral ganglion length, ranked seventeenth and sixteenth in the *covamorph* analysis.

As an overall measure of the relative contributions of size to variance in the 21 variables, one might simply sum the ranks from the *corrmorph* and *covamorph* analyses. Interestingly, the apparent inverse relationship between variance and coefficient of variation caused this summed rank to approximate 21 in most variables. The least size-influenced variable, with a summed rank of 12, was digestive gland length. Also particularly useful were body length, third whorl and aperture width, rostrum width, tentacle length, and radula length. Particularly poor, with a summed rank of 41, was cerebral ganglion width, which showed almost no variance outside the first PC. Also of reduced utility for the same reasons were buccal mass length and width, radula width, and second marginal tooth length.

In sum, it seems that useful measurements can be taken from any aspect of *G. proxima*'s morphology. Hard parts, soft parts, large items and small are all potentially valuable, with scattered exceptions. It seems important, however, that all variables measured in future studies should be of similar size and variance to the extent possible. If very large measurements and very small measurements are combined and a principal component analysis is based on their covariance matrix, variance in the small measurements may be negligible even if the first PC is discarded. If the correlation matrix is factored, the contributions of the large measurements may be negated.

If factor scores from either the *covamorph* or the *corrmorph* analysis are combined with two significant count variables (gill filaments and outer marginal tooth cusps), evidence presented elsewhere suggests that the result-

TABLE 4. Results of principal component analysis on the correlation matrix of 21 *G. proxima* measurements, N = 250.

Variable name	Factor loadings						Rank
	1	2	3	4	5	6	
SHHT	.703	.566	−.039	.016	−.098	.237	16
BWHT	.836	.228	.019	−.012	−.015	.368	21
TWWD	.384	.756	−.051	.047	−.108	−.098	10
APWD	.702	−.139	.327	.121	−.134	.298	13
DGLN	.441	.732	−.168	.003	.075	−.191	8
BDLN	.494	.718	−.170	.019	.094	−.214	7
OPLN	.669	.226	.085	.048	.168	.438	15
RSLN	.576	−.192	−.145	−.397	.243	.062	12
RSWD	.675	−.142	−.148	−.403	−.287	−.262	5
TNLN	.451	−.180	−.096	−.311	.668	.007	4
EYWD	.793	−.085	−.068	−.341	−.128	−.199	18
BMLN	.543	−.217	.200	.156	−.143	−.241	17
BMWD	.757	−.192	.047	.136	−.175	−.262	19
CGLN	.424	−.122	−.091	.670	.312	−.112	2
CGWD	.502	−.021	.167	.323	−.054	−.107	20
PGWD	.271	.014	.537	.046	.217	−.403	6
PDIA	.008	.167	.853	−.248	.078	−.055	1
JWLN	.501	−.303	.462	−.026	−.321	.185	3
RDLN	.640	−.364	−.470	.067	−.202	−.004	9
RDWD	.627	−.366	.243	.079	.281	−.010	14
MGLN	.608	−.526	−.396	.067	.066	.004	11
Eigenvalue—	7.136	2.924	1.977	1.230	1.118	1.025	
Cumulative variance—	.340	.479	.573	.632	.685	.734	

ing measures of overall morphological population divergence between the 25 *G. proxima* populations are significantly positively correlated with a number of other matrices (Dillon, 1984). First, even though the *corrmorph* and *covamorph* measures were based virtually on different sets of variables, they were found to be correlated with each other at the .001 level. Secondly, both matrices were correlated with a matrix of Rogers genetic distances between the 25 populations, calculated from allele frequencies at seven enzyme loci. Thirdly, both *corrmorph* and *covamorph* are correlated with geographic distance between the 25 populations, measured through water or over land. Finally, both matrices are correlated with environmental difference between the 25 populations, estimated using various physical, chemical, and biological variables.

These findings are necessary but not sufficient evidence that morphological data taken on *G. proxima*, analytically treated as it has been here, does in fact have some genetic component. The conclusion that can be drawn with the greatest certainty is that the measures of morphological divergence developed in this analysis are not substantially composed of measurement error.

ACKNOWLEDGEMENTS

Special appreciation is due to my advisor, Dr. George Davis, and my wife, Shary, for indispensable tangible and intangible support, respectively. Bob Hershler showed me much of what I know about dissecting snails. The manuscript was critically reviewed by Dr. Steve Chambers and Dr. John Hendrickson. Dr. R. J. Horwitz, Dr. W. J. Ewens, Dr. R. Ricklefs, and an anonymous reviewer also provided useful advice and criticism. Computer time was provided by the University of Pennsylvania Department of Biology. This research was primarily supported by an NSF grant for improving doctoral dissertation research, No. DEB-8023344.

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