

GENETIC STRUCTURE OF *PILEOLARIA PSEUDOMILITARIS*
(POLYCHAETA: SPIRORBIDAE)

RICHARD BECKWITT

Occidental College, Los Angeles, CA 90041

with Appendix by

RANAJIT CHAKRABORTY

*Center for Demographic and Population Genetics, University of Texas
Health Science Center, Houston, Texas 77025*

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ABSTRACT

The genetic structure of *Pileolaria pseudomilitaris* was studied by means of gene-diversity analysis of allozyme frequencies. At an esterase locus, most of the gene diversity was due to subdivision of the population into colonies and subpopulations separated by less than 100 meters. Gene frequencies at a phosphoglucose isomerase locus were similar over many kilometers, but differed between two habitat types. Differences between colonies are attributed to drift and founder effect; similarities over greater distances are attributed to similar selection pressures. A mathematical appendix details the method of gene diversity analysis for a multi-leveled, hierarchically subdivided population.

AMONG marine invertebrates, some organisms are particularly well suited for a study of genetic structure. In the polychaete family Spirorbidae are a number of species of small, tube-dwelling, filter-feeding worms. Adults are simultaneous hermaphrodites, embryos are brooded and the planktonic larval stage is reduced or absent (GEE 1963; POTSWALD 1968). *Pileolaria pseudomilitaris* (Thiriot-Quievreux) is an intertidal spirorbid that is common on hard substrates in subtropical and tropical areas of the Atlantic and Pacific oceans and the Mediterranean sea (VINE, BAILEY-BROCK and STRAUGHAN 1972). In southern California, the worms live in two distinct habitats: on rocks in mid-intertidal pools and on floating docks and *Mytilus edulis* shells in boat harbors. Gene frequency distributions among local populations of *P. pseudomilitaris* were studied to estimate the degree of population subdivision and the amount of genetic differentiation between populations and between habitat types.

A priori, one might expect *Pileolaria* populations to be highly subdivided. The major means of dispersal between local populations is a very brief planktonic larval stage, although there is a chance that whole populations could be rafted long distances on floating objects or ship hulls. One might also expect large differences between the two habitat types. A tide pool on the rocky outer coast is

different from a floating dock in a protected boat harbor, not only in physical factors such as temperature, water movement and wave shock, but also in biological factors such as available food, predators and competitors. Worms from these two habitats are judged conspecific in terms of traditional morphological characters (P. KNIGHT-JONES, personal communication; BECKWITT 1981) and are interfertile in the laboratory.

NEI (1973; see also CHAKRABORTY 1974) devised a method called gene-diversity analysis to estimate the magnitude of subdivision in a population that consists of semi-isolated subpopulations. CHAKRABORTY (see APPENDIX 1) extended this method to the general case of any number of nested subdivisions. The nature of *P. pseudomilitaris* populations lends them to this type of analysis. The species can be divided into two habitat types; within each habitat type, each intertidal cove or small boat harbor is a separate population. Each population can be divided into several subpopulations, consisting of nearby tidepools or boat slips. Individual tide pools or boat slips are readily identifiable colonies, and each colony can be sampled more than once. The present analysis is based on this five-level hierarchy. Gene-diversity analysis provides a measure of the relative amount of subdivision due to level. From this analysis, one gets a picture of the scale at which differentiation occurs, which suggests hypotheses about the causes of differentiation.

MATERIALS AND METHODS

Standard methods of starch gel electrophoresis were adapted to spirorbid polychaetes. Enzyme stains were taken from SELANDER *et al.* (1971). Two buffers were used: Phosphate (SELANDER *et al.* 1971) and Tris-maleate (GRASSLE and GRASSLE 1976). Seven loci (6 enzyme stains) were scored: Phosphoglucose isomerase (PGI), esterase (EST) and leucine amino peptidase (LAP) were used with the phosphate buffer; phosphoglucomutase (PGM), malate dehydrogenase (MDH) and glutamate oxaloacetic aminotransferase (GOT-1, GOT-2) were used with the tris-maleate buffer. Common alleles at all loci follow Mendelian inheritance in laboratory culture (BECKWITT 1979). A sample collected in the field consisted of about 40 worm tubes, from 1 or a few adjacent intertidal stones or *M. edulis* shells. Live specimens were returned to the laboratory and used for electrophoresis within 1 week. Actual sample sizes were sometimes less than 40, if there were many empty tubes. An individual spirorbid could be stained for 3 enzymes. Therefore, after worms had been removed from their tubes, half of the individuals in a sample were used for each set of enzyme stains.

A total of 21 samples (613 worms) was taken from 2 intertidal coves and 3 small boat harbors in southern California. Royal Palms is a small rocky intertidal cove on the Palos Verdes Peninsula, Los Angeles County, with tide pools in a horizontal rock shelf. One subpopulation at Royal Palms was made up of 5 colonies from small tide pools, all at the same tidal height and separated by less than 10 meters. One or 2 samples were taken from each colony (RPB, RPC, RPD, RPE1, RPE2, RPF1, RPF2). The second subpopulation at Royal Palms was in a large pool about 100 meters away. Three samples were taken from this subpopulation (which consisted of a single colony: RPL1, RPL2, RPL3). Lunada Bay is another rocky cove on the Palos Verdes Peninsula. Two subpopulations were sampled, each located in a single tide pool and separated by about 100 meters. There were 2 samples taken from the first subpopulation (a single colony: LBA1, LBA2), and 1 from the other (LBB). Holiday Harbor is a small boat marina in San Pedro Harbor, Los Angeles Harbor, Los Angeles County. Three samples were taken from a single boat slip or colony (HHA1, HHA2, HHA3). A single sample was taken from a colony in a second subpopulation, a boat slip about 100 meters away (HHB). Marina del Rey is a man-made small boat

harbor, also in Los Angeles County. Two samples were taken from one colony (DRA1, DRA2), and a single sample was taken from the colony in an adjacent boat slip in the same subpopulation about 10 meters away (DRB). A final sample was taken from the small boat harbor in Santa Barbara, Santa Barbara County (SB).

Genetic distance between samples was calculated using the minimum distance measure (D_m) of NEI and ROYCHOUDHURY (1974). This measure was chosen over the variety of similar measures because it is the most conservative of NEI's measures, and there is a statistical test of significance. Also, it is closely related to the measure of gene-diversity analysis used in the subsequent analysis.

Allozyme frequencies were used in the calculation of gene diversity following the method of CHAKRABORTY (see APPENDIX 1), extended to 5 hierarchical levels. Sample gene frequencies were calculated in the usual manner. Gene frequencies for higher levels of subdivision were the average of all samples within that subdivision (not weighted by sample size).

By extension from the case illustrated in the APPENDIX, $H_T = H_Y + D_{YG} + D_{GS} + D_{SP} + D_{PH} + D_{HT}$, where $D_{YG} = H_G - H_Y$ is the gene diversity between samples, and so forth. From equation (1) in the APPENDIX, the relationship between gene diversity and minimum distance is also apparent.

Finally, it is possible to calculate a series of coefficients of gene differentiation (NEI 1973) that estimate the proportion of the total gene diversity that is due to subdivision at each level of the hierarchy. Thus, $G_{GS(T)}$ is the proportion of the total gene diversity that is due to subdivision of the subpopulations into colonies, and so on.

RESULTS

Gene diversity: Table 1 is the matrix of minimum genetic distance (D_m) between all pairs of samples of *Pileolaria pseudomilitaris*. Sample size and gene frequencies for all samples are given in APPENDIX 2. It is apparent that there is appreciable genetic differentiation. Most (200 of 210) pairwise comparisons of samples are significantly different. Even among pairs of samples from single colonies, there are significant differences at six of 10 comparisons. This is striking evidence for genetic subdivision between samples separated by less than 10 meters from seemingly identical habitats, with no apparent barriers to movement. It may be that, among 40 adjacent worms, many are full or half siblings (see description of setting behavior below).

A dendrogram (Figure 1), using D_M as the index, was constructed using a nearest-neighbor sorting strategy (= single-linkage; SOKAL and SNEATH 1963). This sorting strategy is the least likely to form widely separated groups. Even so, the samples sort out into two coherent units: one including all the samples from intertidal areas, and the other all the samples from boat harbors. Within these two large groups there is a tendency for samples from a single population or subpopulation to group together.

The results of the gene diversity analysis are given in Table 2. Gene diversity within samples (average heterozygosity of samples) accounts for about 70% of the total. The remaining 30% is due largely to subdivision by habitat type ($G_{HT} = 9\%$) and population ($G_{PH(T)} = 8\%$). Subdivision at lower levels adds another 4% each. The overall pattern is the same as that seen in the dendrogram.

When data from all loci are averaged together, the major components of differentiation are due to differences between habitats and populations. Alone, this does not indicate whether differentiation is due to selection or isolation. Differ-

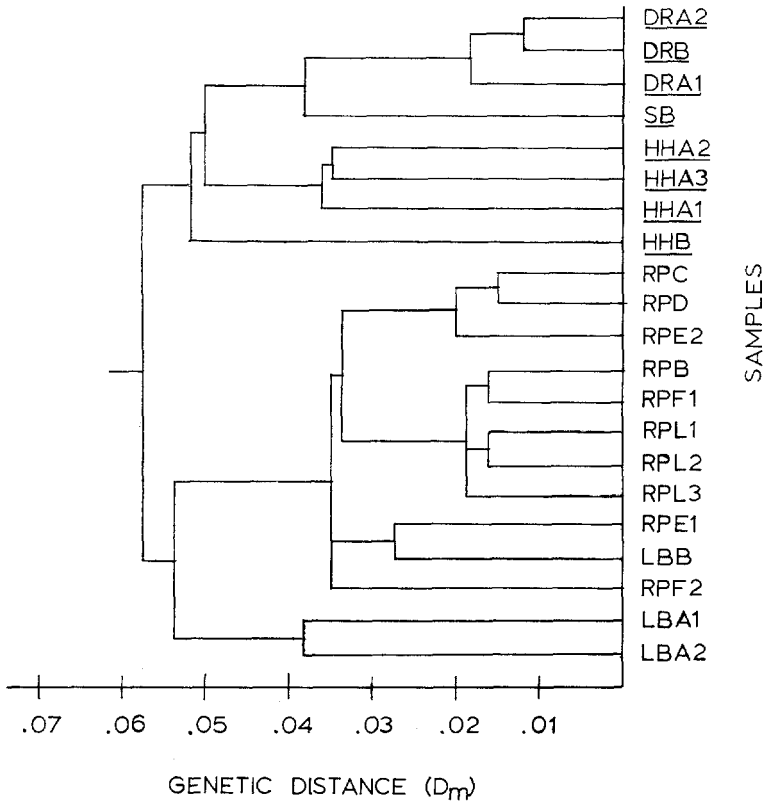


FIGURE 1.—Dendrogram of genetic distance between samples of *Pileolaria pseudomilitaris*, based on all loci. Samples from boat harbors are underlined.

ences among samples or colonies are of lesser magnitude; yet, they too are significant. Note that the standard errors in Table 2 are due to inter-locus variation within one level of the hierarchy, rather than intra-locus variation between colonies, subpopulations, etc.

By examining gene diversity at each polymorphic locus separately, it is possible to decide whether all loci respond in the same way to the environment. Also included in Table 2 are gene diversities for the four polymorphic loci ($H_y > 0.05$). There are striking differences among these loci. Average heterozygosity within samples is comparable at all loci, as is total gene diversity. The partitioning of gene diversity among the levels of the hierarchy, however, is different for each locus.

The gene diversity at the *Pgi* locus is high between habitat types and moderate between populations (16% and 5%, respectively). All of the lower levels contribute only an additional 3%. The *Pgm* locus has a very similar pattern. By contrast, for the *Est* locus, gene diversities between habitats and populations account for only 0.6% and 3%, while subdivision into subpopulations and colonies provides the bulk (24%). In the *Lap* locus, most gene diversity is due to sub-

TABLE 1
*Minimum genetic distance (D_m) for all pairs of samples of *Pileolaria pseudomilitaris**

	Intertidal										Boat Harbor											
	RPC	RPD	RPE1	RPE2	RPE3	RPF1	RPF2	RPF3	RPL1	RPL2	RPL3	LBA1	LBA2	LBB	SB	HHAI	HHA2	HHA3	HFB	DRA1	DRA2	DRB
RPB	0.025*	0.046	0.070	0.034	0.016†	0.084	0.040	0.049	0.057	0.057	0.057	0.099	0.113	0.063	0.068	0.069	0.113	0.088	0.129	0.193	0.177	0.162
RPC		0.015*	0.057	0.029	0.059	0.156	0.092	0.110	0.126	0.126	0.126	0.159	0.170	0.079	0.089	0.085	0.122	0.146	0.206	0.253	0.263	0.200
RPD			0.035	0.020	0.077	0.169	0.106	0.126	0.148	0.148	0.133	0.156	0.156	0.059	0.092	0.106	0.180	0.153	0.214	0.242	0.251	0.187
RPE1				0.037*	0.116	0.123	0.125	0.133	0.169	0.169	0.116	0.142	0.142	0.027†	0.093	0.062	0.143	0.122	0.163	0.168	0.182	0.129
RPE2					0.055	0.099	0.072	0.070	0.084	0.084	0.113	0.148	0.068	0.068	0.106	0.093	0.158	0.119	0.186	0.254	0.246	0.195
RPF1						0.082	0.019	0.028	0.023	0.023	0.096	0.102	0.098	0.100	0.114	0.127	0.106	0.137	0.226	0.267	0.267	0.170
RPF2							0.054	0.035*	0.056	0.056	0.095	0.120	0.133	0.157	0.132	0.145	0.106	0.127	0.271	0.207	0.207	0.202
RPL1								0.016	0.031	0.031	0.054	0.063	0.104	0.103	0.119	0.123	0.096	0.119	0.236	0.173	0.167	0.167
RPL2									0.019	0.019	0.075	0.110	0.137	0.116	0.125	0.140	0.102	0.135	0.263	0.202	0.198	0.198
RPL3											0.128	0.141	0.163	0.162	0.163	0.170	0.128	0.166	0.308	0.247	0.243	0.243
LBA1												0.038*		0.081	0.068	0.131	0.154	0.098	0.099	0.145	0.087	0.087
LBA2													0.062	0.100	0.129	0.128	0.106	0.087	0.096	0.101	0.103	0.086
LBB														0.064	0.057	0.106	0.087	0.096	0.101	0.104	0.064	0.064
SB															0.050	0.050	0.055	0.076	0.084	0.077	0.065	0.038
HHAI																0.036†	0.050	0.061	0.118	0.127	0.127	0.084
HHA2																	0.035†	0.057*	0.159	0.142	0.142	0.125
HHA3																		0.035†	0.057*	0.165	0.124	0.102
HHB																			0.052*	0.092	0.060	0.063
DRA1																				0.023	0.018*	0.012*
DRA2																						

D_m values for all pairs of samples are significantly different from zero ($P < 0.01$) except those marked with an asterisk (*, $P < 0.05$) or a dagger (†, $P > 0.05$).

TABLE 2

Gene diversity analysis of Pileolaria pseudomilitaris

Gene diversity (<i>D</i>)	<i>Pgi</i>	<i>Pgm</i>	<i>Est</i>	<i>Lap</i>	Average	S.E.
H_Y (within samples)	0.540	0.499	0.443	0.433	0.273	0.092
D_{YC} (between samples, within colonies)	0.015	0.023	0.022	0.039	0.015	0.006
D_{CS} (between colonies, within subpopulations)	0.004	0.009	0.068	0.018	0.014	0.009
D_{SP} (between subpopulations, within populations)	0.002	0.011	0.089	0.008	0.016	0.012
D_{PH} (between populations, within habitats)	0.036	0.025	0.022	0.128	0.030	0.017
D_{HT} (between habitats)	0.110	0.071	0.004	0.054	0.034	0.017
H_T (total gene diversity)	0.706	0.590	0.647	0.683	0.382	0.130
<u>Coefficient of gene differentiation (<i>G</i>)</u>						
H_Y/H_T (within samples)	0.765	0.762	0.685	0.634	0.714	0.200
$G_{YC(T)}$ (between samples)	0.021	0.040	0.034	0.058	0.038	0.019
$G_{CS(T)}$ (between colonies)	0.006	0.015	0.104	0.027	0.037	0.023
$G_{SP(T)}$ (between subpopulations)	0.002	0.018	0.138	0.012	0.042	0.029
$G_{PH(T)}$ (between populations)	0.051	0.042	0.033	0.187	0.079	0.034
G_{HT} (between habitats)	0.156	0.121	0.006	0.079	0.089	0.014

Included are data for four polymorphic loci and the average of all seven loci.

division into populations (19%) or habitats (8%), but an appreciable amount is due to differences between samples within colonies (6%).

Differences between loci can also be seen in dendrograms based on single-locus measures of genetic distance. Figures 2a and 2b are dendrograms based on the *Pgi* and *Est* loci, chosen to represent the two extreme types. For the *Pgi* locus, habitat differences are accentuated, and to a lesser extent, population differences are also apparent. For the *Est* locus, on the other hand, there is no discernible pattern. Samples from different populations or habitat types are often joined together as the most similar, and there is no tendency to form groups.

Larval dispersal: Although it is not possible to measure dispersal rates in the field, results of simple behavior experiments in the laboratory indicate that dispersal can be quite low under favorable circumstances. *Pileolaria pseudomilitaris* will settle readily on plastic or glass, among other hard surfaces. One or two adult worms, with brooded embryos, were placed in one quadrant of a 50 mm plastic petri dish in a well-aerated one liter aquarium (particles suspended in the water were carried to all parts of the aquarium within 30 sec). Of the total of 151 larvae, in seven experiments, 120 settled in the dish with their parents and only 31 in other parts of the aquarium. Still more striking, of the 120 that settled in the petri dish, 96 settled in the same quadrant as their parents. Under laboratory conditions, 60% of the larvae settled within 20 mm of their parents. Observations of larvae that hatched normally indicated that most larvae contacted the substrate and explored it immediately. Many metamorphosed without ever swimming (*cf.*, GFE 1963).

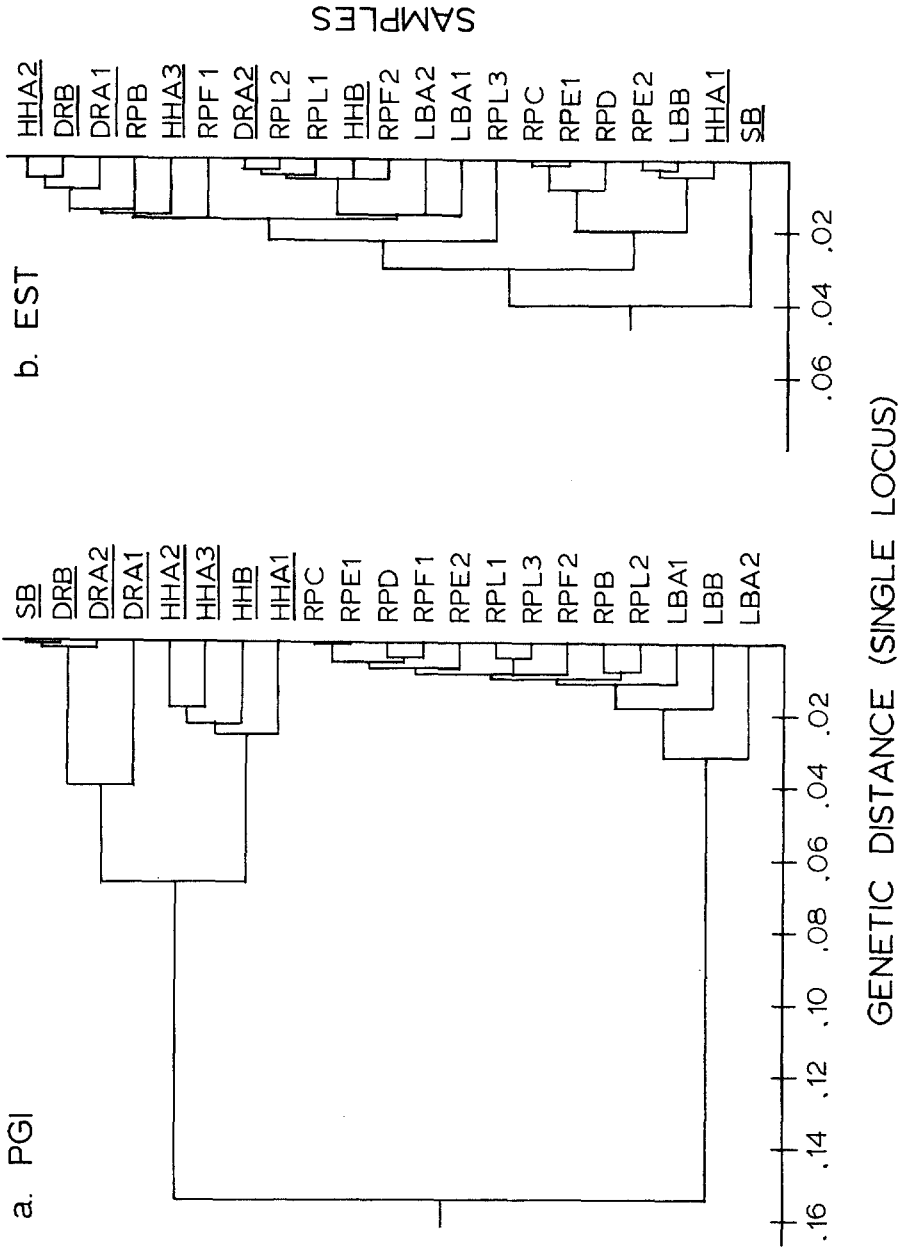


FIGURE 2.—Dendrograms of genetic distance based on single loci. Samples from boat harbors are underlined. A: *Pgi* locus. B: *Est* locus.

DISCUSSION

Pileolaria pseudomilitaris populations are highly subdivided. There are significant differences in gene frequencies between adjacent tidepools or boat slips. Coefficients of gene differentiation, averaged over all loci, indicate that differentiation is greatest between habitat types and nearly as great between populations.

Different enzymatic loci appear differentiated at different geographic scales. AYALA (1974), CHRISTENSEN and FRYDENBERG (1974) and LEVINTON and SUCHANEK (1978) have all argued from similar evidence that natural selection is responsible for some, if not all, of the differences. LEWONTIN and KRAKAUER (1973) suggested comparing the observed variance of F_{ST} with its expected variance. They were criticized (NEI and MARUYAMA 1975; ROBERTSON 1975) for underestimating the expected variance. ROBERTSON has shown how difficult it is to use the variance among loci as evidence for nonneutrality, especially if the population is subdivided in a hierarchical fashion. Using ROBERTSON's method of estimating the added variance due to hierarchical structure, and the average coefficients of gene differentiation as the branch lengths of a tree of relationships among samples (a crude first approximation of the true correlation matrix of gene frequencies, based on a small number of loci), the expected variance among loci of the variance of gene frequencies within samples is $V(V_w) = 0.00726$. The observed value of this variance, calculated as the variance of $D_{YT}/2$ among loci (NEI 1973), is 0.00055, less than the expected variance. By combining samples into colonies and colonies into subpopulations, etc., it is possible to repeat this analysis at each level of the hierarchy. At no level is the observed variance significantly greater than the expected variance; it is not possible to reject the hypothesis that all alleles are neutral.

The larval settlement behavior of spirorbid has been well studied in several species. Most of the species studied are gregarious; that is, they are stimulated to settle by the presence of others of the same species (KNIGHT-JONES 1951). Among species that are restricted to a limited range of algae as preferred substrate, settlement can be delayed in the absence of the appropriate stimulus (DE SILVA 1962; GEE and KNIGHT-JONES 1962). *Pileolaria pseudomilitaris* differs from most of the species studied in that it is not found on a restricted range of substrates. Larvae are neither selective nor loyal (in the sense of DOYLE 1976). Under natural conditions, even selective spirorbid species may have an abbreviated larval stage, since they are released into the environment with a great likelihood of contacting a favorable site immediately (GEE 1963). Larvae may decline to settle if the parent population is crowded. WISELY (1960) has shown that the gregarious response of *Spirorbis borealis* is inhibited when densities exceed about 10/cm². In the laboratory, the maximum observed densities of *P. pseudomilitaris* approximated this value (10.8/cm²). Dispersal may be nil in moderate populations, but may increase as density increases.

Spirorbid colonies appear to be transitory. Intertidal rocks are often overturned, and the undersides of such rocks are covered with empty spirorbid tubes. On *M. edulis* shells, *P. pseudomilitaris* is apparently overgrown by encrusting sponges and bryozoans. Under such circumstances, founder effect can cause colonies to differ, in spite of moderate selection.

The genetic structure of *Pileolaria pseudomilitaris* can be hypothetically reconstructed as follows: Each population is a mosaic of semi-isolated colonies. Demes consist of a single tidepool or boat slip, or at most they extend a few tens of meters. Colonies are subject to a high rate of extinction, and are replaced by a

few founder individuals. Most of the offspring remain within a colony; thus, populations can increase rapidly. The few larvae that are transported beyond colony boundaries are not sufficient to keep colonies genetically uniform, but do form a pool to initiate new colonies.

If *Pileolaria pseudomilitaris* has the type of genetic structure I have suggested, it is difficult to explain the large number of alleles segregating in each colony. High extinction rates and small effective population size are factors that should decrease heterozygosity and number of alleles. Yet, all polymorphic loci typically have three to five alleles in a single sample. Although spirorbids are capable of self-fertilization (GEE and WILLIAMS 1965; POTSWALD 1968; BECKWITT 1979), most colonies can not be the isolated progeny of a single individual. Gene flow between colonies is apparently sufficient to introduce new alleles, but not sufficient to keep gene frequencies similar.

The genetic structure of *P. pseudomilitaris* appears to be more highly subdivided than other marine invertebrates that have been studied. GOOCH (1975), in an extensive review, has tabulated the relationship between dispersal ability and genetic subdivision. In general, there is an increasing trend towards regional differentiations in species with limited dispersal, although individual loci may respond in opposite fashion. LEVINTON and SUCHANEK (1978) concluded that large amounts of gene flow keep *M. californianus* populations similar when there is little environmental heterogeneity, but they emphasize the significance of microhabitat differences. GRASSLE and GRASSLE (1977, 1978) have discussed the genetic consequences of dispersal, population size and environmental instability, with particular reference to the polychaete *Capitella*. They suggest that opportunistic species, which are less well dispersed, are more likely to be divided into subpopulations because of short-term selection.

Pileolaria pseudomilitaris has many of the properties of an opportunistic species, including reduced dispersal, moderate genetic variability and highly subdivided populations. Gene-diversity analysis cannot provide much information about the relative significance of selectionist or neutralist causes of this subdivision. Intuitively, one might expect differences between habitat types to reflect the significant environmental differences between a small boat harbor and a tide-pool. Such a hypothesis must be tested by transplants or other experimental manipulations. However, there is considerable genetic subdivision, even within one habitat type, from adjacent colonies or samples. Subdivision at this small scale is more likely due to reduced dispersal, leading to inbreeding within colonies. Genetic structure at this scale is more likely to represent peculiarities in the biology of a particular species. The colony by colony pattern of gene frequencies is not likely to be stable over many generations. Differences in gene frequencies, although statistically significant, relate more to reproductive events within one generation than to processes of adaptation or speciation that act over many generations.

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Corresponding editor: M. NEI

APPENDIX 1

GENE-DIVERSITY ANALYSIS IN NESTED SUBDIVIDED POPULATIONS

RANAJIT CHAKRABORTY

Center for Demographic and Population Genetics, University of Texas Health Science Center, Houston, Texas 77025

NEI (1973) proposed a method of decomposing the gene diversity (heterozygosity) of a population into its components, *i.e.*, the gene diversities within and between subpopulations. He also indicated that this method can be extended to the case where each subdivision is further subdivided, but did not show the details of the procedure. I present below the method of gene-diversity analysis when a population is divided into s subpopulations and the i th subpopulation ($i = 1, 2, \dots, s$) is composed of c_i colonies. Let $c = \sum_{i=1}^s c_i$ be the total number of colonies in the entire population. In the accompanying paper, the same method is applied to describe the population structure of a small marine polychaete worm (*Pileolaria pseudomilitaris*) with five levels of nested hierarchical division. To illustrate the method, I shall simply consider the *Pgi* gene frequency data and two levels of subdivision "species to habitats to populations" from the accompanying paper.

Let x_{ijk} represent the frequency of the k th allele at a locus in the j th colony of the i th subpopulation ($i = 1, 2, \dots, s; j = 1, 2, \dots, c_i$). The frequency of the same allele in the i th subpopulation is then given by

$$x_{i \cdot k} = \frac{1}{c_i} \sum_{j=1}^{c_i} x_{ijk},$$

and the frequency of this allele in the entire population is

$$\begin{aligned} x_{\cdot \cdot k} &= \frac{1}{c} \sum_{i=1}^s c_i x_{i \cdot k} \\ &= \frac{1}{c} \sum_{i=1}^s \sum_{j=1}^{c_i} x_{ijk}. \end{aligned}$$

The gene identity values in the entire population, i th subpopulation, and j th colony of the i th subpopulation are then given by

$$\begin{aligned} J_T &= \sum_k x_{\cdot \cdot k}^2, \\ J_i &= \sum_k x_{i \cdot k}^2. \end{aligned}$$

and

$$J_{ij} = \frac{\sum_k x_{ijk}^2}{k},$$

respectively.

The quantity J_T may be written as

$$\begin{aligned} J_T &= \frac{1}{k} \left(\frac{1}{c} \sum_i c_i x_{i \cdot k} \right)^2 \\ &= \frac{1}{c^2} \left[\sum_i c_i^2 \left(\frac{\sum_k x_{i \cdot k}^2}{k} \right) + \sum_{i \neq i'} c_i c_{i'} \left(\frac{\sum_k x_{i \cdot k} x_{i' \cdot k}}{k} \right) \right] \\ &= \frac{1}{c} \sum_i c_i J_i - \frac{1}{c^2} \sum_{i \neq i'} c_i c_{i'} D_{i i'}, \end{aligned} \quad (1)$$

where $D_{i i'} = H_{i i'} - (H_i + H_{i'})/2$ is the gene diversity between the i th and i' th subpopulations; $H_i = 1 - J_i$; and $H_{i i'} = 1 - \frac{\sum_k x_{i \cdot k} x_{i' \cdot k}}{k}$.

Note that the first term in (1) describes the average gene identity within subpopulations (J_S), and the second term represents the average gene diversity between subpopulations (D_{ST}). We thus have

$$J_T = J_S - D_{ST},$$

which is equivalent to NEI's equation [7] (NEI 1973).

To decompose the within-subpopulation gene identity (J_S) into its components considering the subdivision into colonies, we write

$$\begin{aligned} J_S &= \frac{1}{c} \sum_i c_i J_i \\ &= \frac{1}{c} \sum_i c_i \sum_k \left[\frac{1}{c_i} \sum_j x_{ijk} \right]^2 \\ &= \frac{1}{c} \sum_i \left[\sum_j J_{ij} - \frac{1}{c} \sum_{j \neq j'} D_{ij, ij'} \right] \\ &= \frac{1}{c} \sum_{ij} J_{ij} - \frac{1}{c} \sum_i c_i D_{CS_i}, \end{aligned} \quad (3)$$

where $D_{CS_i} = 1/(c^2) \sum_{j \neq j'} D_{ij, ij'}$ is the average gene diversity between colonies within the i th subpopulation. Clearly, the first term of (3) represents the average gene identity within all colonies (J_C), and the second term is the average gene diversity between all colonies within subpopulations (D_{CS}). Combining (2) and (3) we thus have

$$H_T = H_C + D_{ST} + D_{CS}, \quad (4)$$

which gives the same equation that NEI (1973) derives for a hierarchial population structure. It may be mentioned that, for any number of loci, we need only replace the gene diversity for a locus by the average gene diversity for all loci studied. Thus, we have

$$\begin{aligned} H_C &= 1 - \sum_{ij} J_{ij}/c, \\ D_{ST} &= H_T - H_S = J_S - J_T \end{aligned}$$

and

$$D_{CS} = H_S - H_C = J_C - J_S.$$

As an example, let us consider the 21 sampling sites of the population of *P. pseudomilitaris* and the gene frequencies at the *Pgi* locus, which may be computed from the table of APPENDIX 2. The computed gene frequencies for the two levels of subdivision of the whole species data into habitats and populations together with the number of sampling sites within each unit is shown

TABLE A1

Gene frequencies and gene-diversity statistics at Pgi locus of P. pseudomilitaris at population, habitat and species level

Levels of subdivision	Units	No. of sampling sites	Allele frequency								Gene identity (J)
			<i>pgi</i> ⁻¹⁰	<i>pgi</i> ⁻⁵	<i>pgi</i> ¹	<i>pgi</i> ⁵	<i>pgi</i> ¹⁰	<i>pgi</i> ¹⁵	<i>pgi</i> ²⁰	<i>pgi</i> ³⁰	
Population	RP	10	0	0	0.272	0.069	0.015	0.030	0.604	0.010	0.4447
	LB	3	0	0	0.238	0.032	0.090	0.175	0.465	0	0.3125
	SB	1	0	0	0.767	0.233	0	0	0	0	0.6422
	HH	4	0.046	0.183	0.342	0.233	0.196	0	0	0	0.2452
	DR	3	0	0	0.850	0.142	0	0.008	0	0	0.7426
Habitat	INT	13	0	0	0.264	0.061	0.032	0.063	0.572	0.008	0.4056
	HARB	8	0.023	0.092	0.585	0.199	0.098	0.003	0	0	0.4008
Species		21	0.009	0.035	0.387	0.113	0.057	0.040	0.354	0.005	0.2938

See accompanying paper for abbreviations.

in Table A1. For the whole species, the gene-identity at the *Pgi* locus is thus given by $J_T = (0.009)^2 + \dots + (0.005)^2 = 0.2938$. The average identity within habitats is computed from Table A1 as

$$J_H = \frac{1}{21} (13 \times 0.4056 + 8 \times 0.4008) = 0.4038 ,$$

and thus D_{HT} (analogous to D_{ST} of the text) $= H_T - H_S = J_H - J_T = 0.1100$.

The average gene identity within the five populations is similarly given by

$$J_P = \frac{1}{21} (10 \times 0.4447 + \dots + 3 \times 0.7426) = 0.4398 ,$$

so that the average gene identity between populations within habitats is

$$D_{PH} \text{ (analogous to } D_{CS} \text{ of text)} = H_H - H_P = J_P - J_H = 0.0360 .$$

We thus have,

$$H_T = H_P + D_{HP} + D_{PH} (0.7062) = (0.5602) + (0.1100) + (0.0360)$$

In the accompanying paper, further subdivision of H_P is done by the hierarchical subdivisions of each populations into subpopulations, colonies and sampling sites.

Finally, it may be noted that, in this note, all sampling sites (called colonies in the text) are taken to be of the same size). In practice, the variation in their population sizes may have to be taken into account, which can be incorporated as described in CHAKRABORTY (1974).

LITERATURE CITED

CHAKRABORTY, R., 1974 A note on Nei's measure of gene diversity in a substructured population. *Humangenetik* **21**: 85-88.
 NEI, M., 1973 Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U.S.* **70**: 3321-3323.

APPENDIX 2

Gene frequency data for all samples of *Pileolaria pseudomilitaris* are provided in the following tables.

TABLE A2
Allele frequencies for Pgi locus of Pileolaria pseudomilitaris

Sample site	Sample size	Allele							
		<i>pgi⁻¹⁰</i>	<i>pgi⁻⁶</i>	<i>pgi¹</i>	<i>pgi⁵</i>	<i>pgi¹⁰</i>	<i>pgi¹⁵</i>	<i>pgi²⁰</i>	<i>pgi⁵⁰</i>
RPB	20	0	0	16	4	0	0	20	0
RPC	20	0	0	12	4	0	0	24	0
RPD	14	0	0	8	0	0	0	20	0
RPE1	10	0	0	6	2	0	0	12	0
RPE2	10	0	0	5	0	2	0	13	0
RPF1	14	0	0	9	0	0	1	18	0
RPF2	9	0	0	3	3	0	2	10	0
RPL1	20	0	0	7	3	2	1	25	2
RPL2	20	0	0	13	2	0	3	20	2
RPL3	20	0	0	8	4	0	2	26	0
LBA1	9	0	0	8	0	1	1	8	0
LBA2	11	0	0	1	1	2	7	11	0
LBB	20	0	0	9	2	5	6	18	0
SB	15	0	0	23	7	0	0	0	0
HHA1	18	1	2	16	11	6	0	0	0
HHA2	5	0	2	3	2	3	0	0	0
HHA3	9	1	5	4	5	3	0	0	0
HHB	10	2	4	8	3	3	0	0	0
DRA1	15	0	0	30	0	0	0	0	0
DRA2	20	0	0	32	8	0	0	0	0
DRB	20	0	0	30	9	0	1	0	0

See text for abbreviations.

TABLE A3
Allele frequencies for Pgm locus of Pileolaria pseudomilitaris

Sample site	Sample size	Allele			
		<i>pgm⁴⁰</i>	<i>pgm⁴⁶</i>	<i>pgm⁵⁰</i>	<i>pgm⁵⁴</i>
RPB	19	0	0	25	13
RPC	19	0	2	25	11
RPD	14	2	0	22	4
RPE1	10	1	6	13	0
RPE2	10	0	0	20	0
RPF1	14	1	1	21	5
RPF2	13	0	1	22	3
RPL1	20	0	2	30	8
RPL2	20	0	1	39	0
RPL3	20	0	1	39	0
LBA1	9	1	1	14	2
LBA2	11	1	8	9	4
LBB	20	1	13	18	8
SB	15	2	6	17	5

TABLE A3—Continued

Sample site	Sample size	Allele			
		<i>pgm⁴⁰</i>	<i>pgm⁴⁸</i>	<i>pgm⁵⁰</i>	<i>pgm⁵⁴</i>
HHA1	14	4	12	10	2
HHA2	6	0	6	3	3
HHA3	7	1	2	9	2
HHB	10	8	8	3	1
DRA1	15	1	22	3	4
DRA2	20	3	23	10	6
DRB	20	3	18	10	9

See text for abbreviations.

TABLE A4

Allele frequencies for Est locus of Pileolaria pseudomilitaris

Sample site	Sample size	Allele			
		<i>est⁵⁸</i>	<i>est⁵⁹</i>	<i>est⁶⁰</i>	<i>est⁶⁶</i>
RPB	20	0	0	20	20
RPC	20	0	0	4	36
RPD	14	0	1	0	27
RPE1	10	0	0	2	18
RPE2	10	0	0	5	15
RPF1	13	0	2	18	6
RPF2	9	0	4	14	0
RPL1	20	0	17	23	0
RPL2	18	0	12	24	0
RPL3	20	0	3	37	0
LBA1	9	0	12	6	0
LBA2	11	0	12	10	0
LBB	19	0	1	11	26
SB	15	7	6	6	11
HHA1	17	2	1	10	21
HHA2	5	0	2	5	3
HHA3	9	2	2	10	4
HHB	10	0	5	14	1
DRA1	15	1	3	15	11
DRA2	20	1	14	24	1
DRB	20	3	8	17	12

See text for abbreviations.

TABLE A5

Allele frequencies for Lap locus of Pileolaria pseudomilitaris

Sample site	Sample size	Allele				
		<i>lap⁸⁷</i>	<i>lap⁸⁹</i>	<i>lap⁹⁵</i>	<i>lap¹⁰</i>	<i>lap¹⁴</i>
RPB	17	0	5	23	6	0
RPC	20	0	4	32	4	0
RPD	14	0	2	16	10	0
RPE1	7	0	6	2	6	0
RPE2	10	0	5	10	4	1

TABLE A5—Continued

Sample site	Sample size	Allele				
		<i>lap</i> ²⁷	<i>lap</i> ³⁰	<i>lap</i> ³⁶	<i>lap</i> ⁴⁰	<i>lap</i> ⁴⁴
RPF1	8	0	2	13	3	0
RPF2	8	0	12	3	1	0
RPL1	19	0	5	25	8	0
RPL2	18	0	12	23	1	0
RPL3	16	0	6	25	1	0
LBA1	7	0	2	2	10	0
LBA2	11	0	0	5	17	0
LBB	19	1	5	5	27	0
SB	13	0	0	10	15	1
HHA1	18	9	10	11	6	0
HHA2	5	6	1	3	0	0
HHA3	9	10	1	2	5	0
HHB	10	5	4	2	9	0
DRA1	15	0	0	0	30	0
DRA2	20	0	0	0	40	0
DRB	20	0	0	0	38	2

See text for abbreviations.

TABLE A6

Allele frequencies for Mdh, Got-1 and Got-2 loci of Pileolaria pseudomilitaris

Sample site	Sample size	Locus and allele				
		<i>mdh</i> ²⁸	<i>mdh</i> ⁴³	<i>mdh</i> ⁴⁸	<i>got-1</i> ¹⁷	<i>got-2</i> ¹⁰
RPB	20	0	40	0	40	40
RPC	20	1	39	0	40	40
RPD	14	0	28	0	28	28
RPE1	10	0	20	0	20	20
RPE2	10	0	18	2	20	20
RPF1	14	0	28	0	28	28
RPF2	13	2	24	0	26	26
RPL1	20	0	40	0	40	40
RPL2	20	0	40	0	40	40
RPL3	20	3	33	4	40	40
LBA1	9	0	18	0	18	18
LBA2	11	0	22	0	22	22
LBB	20	0	40	0	40	40
SB	15	0	30	0	30	30
HHA1	14	0	28	0	28	28
HHA2	6	0	12	0	12	12
HHA3	7	0	14	0	14	14
HHB	10	0	18	2	20	20
DRA1	15	0	30	0	30	30
DRA2	20	1	39	0	40	40
DRB	20	0	40	0	40	40

See text for abbreviations.