

Genetic Structure of Populations and Differentiation in Forest Trees¹

Raymond P. Guries² and F. Thomas Ledig³

Abstract: Electrophoretic techniques permit population biologists to analyze genetic structure of natural populations by using large numbers of allozyme loci. Several methods of analysis have been applied to allozyme data, including chi-square contingency tests, F-statistics, and genetic distance. This paper compares such statistics for pitch pine (*Pinus rigida* Mill.) with those gathered for other plants and animals. On the basis of these comparisons, we conclude that pitch pine shows significant differentiation across its range, but appears to be less differentiated than many other organisms. Data for other forest trees indicate that they, in general, conform to the pitch pine model. An open breeding system and a long life cycle probably are responsible for the limited differentiation observed in forest trees. These conclusions pertain only to variation at allozyme loci, a class that may be predominantly neutral with respect to adaptation, although gene frequencies for some loci were correlated with climatic variables.

During the last 40 years, a considerable body of information has been assembled on genetic variability in forest tree species. Measurements of growth rate, cold-hardiness, phenology, and related traits have provided tree breeders with much useful knowledge, and have indicated in a general way how tree species have adapted to a spatially variable habitat (Wright 1976). Most information has come from studies designed to assess relative differences in metric traits among provenances representing a wide geographic range. When the identification of suitable seed sources for use in reforestation is a primary objective, provenance tests (that is, common garden studies) may be indispensable. However, such studies are expensive to conduct, require large areas of land, yield useful data only after many years, and use traits of unknown inheritance.

The development of electrophoretic techniques during the last two decades provided an alternative to common garden techniques for estimating levels of genetic variation in natural populations (Lewontin 1974). Electrophoretic techniques are now widely used in studies of plant and animal populations and have produced a large body of data on the levels and patterns of genic variability characteristic of many species. Although the difficulties inherent in the large size and late reproductive maturity of trees somewhat delayed their study, there is in recent years a rapid accumulation of useful data from the application of electrophoretic techniques to several conifers.

POPULATION STRUCTURE

A common notion among foresters is that most tree species are divided into relatively small breeding populations because of limited pollen or seed dispersal. Most matings are considered to be among near neighbors, leading in time to the division of a large population into numerous, small "neighborhoods." Other factors such as physical isolation, breeding system, population demography, natural selection, and random genetic drift accentuate such subdivision. The sum total of the ecological and genetic relationships among individuals and the populations they comprise is termed "population structure" (Jain 1975).

Of special interest to tree breeders are the genetic consequences of population structure. Several measures of the genetic structure of populations have been proposed, including assessments of (a) gene diversity in the average population, (b) levels of diversity in different populations, and (c) degree of differentiation among populations (Brown 1978). All these measures are amenable to allozyme analysis, and recent explorations have begun to probe the genetic structure of forest trees.

Our approach in this paper is to compare estimates of various parameters of population structure obtained from pitch pine (*Pinus rigida* Mill.) with those of other species. Rigorous comparisons are seldom possible because of variation in methods of data collection and analysis; therefore, we use them here only for purposes of general description.

CHI-SQUARE ANALYSIS OF HETEROGENEITY

The subdivision of populations may lead to a heterogeneity of gene frequencies among subpopulations as a result of selection or drift. Variation in genic proportions

¹Presented at the Symposium on Isozymes of North American Forest Trees and Forest Insects, July 27, 1979, Berkeley, Calif.

²Assistant Professor of Forestry, University of Wisconsin, Madison, Wisc.

³Project Leader, Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley, Calif.; and Professor of Forest Genetics, Yale University, New Haven, Conn.

can be subjected to a contingency chi-square analysis to determine the existence or extent of any heterogeneity (Workman and Niswander 1970). A major advantage of the technique is ease of calculation; however, the analysis does not identify the forces responsible for any observed heterogeneity. For pitch pine, chi-square was calculated using the technique of Snedecor and Irwin (1933):

$$\chi^2 = 2N \sum_i \frac{\sigma_i^2}{\bar{p}_i}$$

in which N is the total number of individuals, σ_i^2 is the variance in allele frequency, and $\bar{p}_i$ is the weighted mean frequency.

A significant amount of heterogeneity is apparent at most of 21 loci in 11 populations of pitch pine (table 1). Considering the wide range over which pitch pine samples were collected (from Quebec to North Carolina), differential selection in varying environments is one possible explanation. Distribution of allele frequencies was observed to be heterogeneous over relatively short distances in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), perhaps because of differential selection on slopes of varying aspect (Mitton and others 1977). However, Neale (1978) found no evidence of allelic heterogeneity among populations of balsam fir (*Abies balsamea* [L.] Mill.) occurring along an elevational gradient in New Hampshire, although differences in growth and physiological processes were observed and related to environmental variation along the same transect (Fryer and Ledig 1972).

Genetic drift could also produce heterogeneity. In pitch pine, certain populations (for example, one in St. Chryso-

stome, Quebec) are isolated from the main body of the species range. If such populations were founded by a small number of individuals, genetic drift could account for much of the observed heterogeneity. Genetic drift was a possible explanation for observed heterogeneity of gene frequencies among three small populations of Table Mountain pine (*Pinus pungens* Lamb.) in Virginia (Feret 1974). In any event, the large range in chi-square values among loci in pitch pine suggests that different loci are probably responding independently to whatever factors are responsible for the heterogeneity.

F-STATISTIC ANALYSIS OF POPULATION STRUCTURE

The structure of a subdivided population can also be analyzed by F-statistics (Wright 1951, 1965, 1969; Kirby 1975; Nei 1977). F-statistics were originally devised to examine structuring in hierarchical populations by using the correlation between uniting gametes within and among subpopulations and for the population as a whole. Wright (1965) advanced three parameters "in terms of a total population (T), subdivisions (S), and individuals (I). F_{IT} is the correlation between gametes that unite to produce the individuals, relative to the gametes of the total population. F_{IS} is the average overall subdivisions of the correlation between uniting gametes relative to those of their subdivision. F_{ST} is the correlation between random gametes within subdivisions, relative to gametes of the total population. The list can be extended if there are further subdivisions. The above three F-statistics are not independent."

These statistics have as a common focal point the fixation index, F, which represents the total deviation from Hardy-Weinberg proportions because of the joint effects of finite population size, selection, inbreeding, and other factors. The estimate of F_{ISi} for the i^{th} subpopulation was calculated as:

$$F_{ISi} = 1 - H_i / \frac{1}{(1 + 2N_i - 1)} 2p_i q_i$$

in which H_i is the observed number of heterozygotes in the i^{th} subpopulation and the denominator is the expected number corrected for finite population size (Kirby 1975).

F_{IS} represents the average deviation of the population's genotypic proportions from Hardy-Weinberg equilibrium for a locus and is calculated as the weighted mean of the F values for all populations;

$$\hat{F}_{IS} = \sum_i \frac{N_i}{N} p_i q_i F_{ISi} / \sum_i \frac{N_i}{N} p_i q_i$$

A negative F_{IS} value represents an excess of heterozygotes. It is worth noting that F_{IS} not equivalent to the coefficient of inbreeding except in the unlikely event that inbreeding

Table 1—Chi-square contingency analysis of gene frequency heterogeneity by locus for 11 pitch pine populations

Locus	X ²	df	P<
MDH-1	45.76	10	0.005
MDH-2	107.77	10	.005
IDH	95.43	10	.005
FUM	18.22	10	.1
PGM-1	9.39	10	.5
PGM-2	42.22	10	.005
GPI-1	16.60	10	.1
GPI-2	28.07	10	.005
6-PGD-1	166.36	10	.005
6-PGD-2	52.58	10	.005
G-6-P	39.66	10	.005
LAM	51.91	10	.005
LAP-2	37.97	10	.005
GOT-1	44.39	10	.005
GOT-2	34.59	10	.005
ACP	54.92	10	.005
ACO	131.04	10	.005
GDH	31.72	10	.005
ADH	3.64	10	.95
ALD-1	30.00	10	.005
ALD-2	148.57	10	.005

alone is responsible for departures from Hardy-Weinberg equilibrium. F_{IS} can be averaged across all loci.

The extent of differentiation among subpopulations is measured by F_{ST} , the correlation between random gametes within subdivisions, and was calculated as Nei's (1975) G_{ST} :

$$G_{ST} = D_{ST} / H_T,$$

in which H_T is the gene diversity in the total population, D_{ST} is the average gene diversity among subpopulations.

$$H_T = 1 - \sum_k \left(\sum_i p_{ik} / S \right)^2, \text{ and}$$

$$D_{ST} = \sum_i \left(\sum_k p_{ik}^2 \right) - \sum_k \left(\sum_i p_{ik} / S \right)^2.$$

in which p_{ik} is the frequency of the k^{th} allele in the i^{th} subpopulation and S is the number of subpopulations.

For gene loci with only two alleles, F_{ST} was also calculated as:

$$F_{ST} = \sigma_p^2 / \bar{p}\bar{q},$$

in which σ_p^2 is the weighted sum of squared deviations of the individual subpopulation gene frequencies from the mean gene frequency, divided by the number of subpopulations, and P and q represent weighted mean frequencies.

F_{ST} was extended to multiple allele systems by Nei (1965):

$$F_{ST} = -\sigma_{jk} / \bar{p}_j \bar{p}_k,$$

in which

$$\sigma_{jk} = \sum_i \frac{N_i}{N} (p_{ji} - \bar{p}_j)(p_{ki} - \bar{p}_k),$$

$$\bar{p}_j = \sum_i \frac{N_i}{N} p_{ji}, \text{ and}$$

$$\bar{p}_k = \sum_i \frac{N_i}{N} p_{ki},$$

Table 2— F -Statistics from plant, animal, and human populations

Groups of plant, animal, and human populations	Loci	F_{IS}	F_{ST}	F_{IT}	Sampling unit	Source
Japanese	1	0.002	0.001	0.003	All Japan	Nei and Imaizumi 1966
Monarch butterfly	6	.008	.009	.017	Eastern United States	Eanes and Koehn 1978
Balsam fir	7	.001	.012	.013	New Hampshire mountainside	Neale 1978
Papago Indians	7	-.005	.023	.009	10 reservation districts	Workman and Niswander 1970
Pitch pine	21	.009	.024	.034	Eastern United States	Guries and Ledig (in preparation)
Bluegill	3	.012	.029	.024	Within reservoirs	Awise and Felley 1979
Yanomama Indians	8	.022	.064	.045	37 villages in Venezuela	Neel and Ward 1972
Cylindric blazing-star	15	.407	.069	.426	1/8 acre	Schaal 1975
Phlox (3 species)	4 to 6	.503	.200	.643	Central Texas	Levin 1978

where p_{ji} and p_{ki} are the j^{th} and k^{th} alleles in the i^{th} population. F_{ST} for multiple alleles must be calculated for each combination of alleles. For pitch pine, differences between Nei's G_{ST} and Wright's F_{ST} were negligible, and Nei's calculation was preferred.

The overall fixation index, F_{IT} , represents the correlation between uniting gametes relative to the gametes of the total population and was calculated from F_{IS} and F_{ST} as:

$$F_{IT} = F_{IS} + (1 - F_{IS}) F_{ST}.$$

Like F_{IS} , F_{IT} may be positive or negative, with a negative value indicative of excess heterozygotes. If all populations are in Hardy-Weinberg equilibrium, $F_{IS} = 0$ and $F_{IT} = F_{ST}$. However, even if Hardy-Weinberg proportions are obtained, differentiation because of differing allele frequencies in subpopulations can lead to significant F_{ST} and F_{IT} values.

F -statistics are arranged by increasing F_{ST} values for several species of plants and animals including human populations in table 2. Two species of trees, pitch pine and balsam fir, for which estimates are included in table 2, were both characterized by relatively small F_{IS} values, an indication that populations are at or near Hardy-Weinberg equilibrium. This may or may not indicate that inbreeding is insignificant, because a tendency toward increasing F_{IS} values by way of inbreeding could be offset by such factors as migration or differential fertility. In fact, opposing forces probably counter-balance each other in many instances, with the net result that F (and, therefore, F_{IS}) is near zero (Workman 1969).

For both tree species, F_{ST} values were also small, relative to other organisms. Balsam fir, which did not show allelic heterogeneity in contingency chi-square tests (Neale 1978), has a value of F_{ST} about one-half as large as pitch pine. However, the range sampled in pitch pine was much larger, more than 1000 km in both north-south and east-west directions, while balsam fir was sampled on a single mountainside. F_{ST} values have been calculated for a variety of other organisms not given in table 2, but including the house mouse ($F_{ST} = 0.024$, one barn; Selander 1970), *Drosophila robusta* ($F_{ST} = 0.055$, Eastern United States; Prakash 1973), and the brown snail ($F_{ST} = 0.116$, a city

block; Selander and Kaufman 1975). Geographic differentiation for pitch pine across its range is roughly no larger than that observed among bluegill populations within a single lake or Indian settlements on a single Indian reservation. The estimates for pitch pine are considerably smaller than those obtained for brown snails within the narrow confines of a city block or the herbaceous plant, cylindric blazing-star (*Liatris cylindracea* Michx.), growing on a plot of only 1/8-acre. Although population differentiation has occurred in pitch pine as indicated by the chi-square contingency test, it has not developed to the degree characteristic of many other organisms.

As a result of a low fixation index within populations (low F_{IS}) and a lack of extensive differentiation among populations (low F_{ST}), the total fixation index, F_{IT} , in pitch pine is also low. The two estimates for herbaceous plants included in table 2 have F_{IT} values from 10 to 20 times greater than pitch pine, a feature probably indicative of a high degree of inbreeding in the herbs.

ANALYSIS OF GENETIC DISTANCE

Perhaps the most widely applied index of genetic differentiation is that of genetic distance (Nei 1972). This method was developed to use isozyme data as a measure of the accumulated number of gene substitutions per locus and is defined by:

$$D = -\log_e I$$

in which I represents the normalized identity of genes. If two populations have the same alleles in the same frequency, $I = 1$; when two populations have no alleles in common, $I = 0$.

Estimates of genetic distance among populations within a species (or among species) are available for a number of plants and animals (see reviews of Avise 1974, Gottlieb 1977). Estimates of the mean genetic distance among conspecific populations for a number of species indicate that divergence among populations of tree species has been rather limited relative to that found in other organisms over much smaller areas (table 3). Results are consistent

with F_{ST} values presented earlier. It should be noted that all tree species for which such estimates are available are outbreeding and anemophilous, and gene flow may be substantially greater than for trees that use animals as pollen vectors.

Comparisons of genetic distance with geographic distance have frequently been useful in determining whether differentiation can be explained on the basis of isolation by distance. For example, genetic distance in cylindric blazing-star (table 2) was strongly correlated with geographic distance and "neighborhoods" became well-differentiated over distances of several meters (Schaal 1974).

To test whether a significant relationship exists between genetic and geographic distance for populations of pitch pine, a product-moment correlation was calculated and proved to be nonsignificant ($r = 0.263$; $0.1 > P > 0.05$). By contrast, a significant correlation was noted between genetic and geographic distance for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) populations in coastal British Columbia.⁴ However, it appears that much of the differentiation observed in Douglas-fir was the result of differences between island and mainland populations.⁵

RELATIONSHIP TO ENVIRONMENTAL GRADIENTS

Geographic distance *per se* may not be the most useful parameter by which to measure isolation, especially if the loci in question are under selection pressure. Therefore, product-moment correlations between the frequency of the most common allele and several climatic variables were calculated for the 11 most polymorphic loci in pitch pine (table 4).

Significant correlations were noted for six of the 11 loci. Although such correlations are not evidence for the operation of natural selection, they parallel clinal patterns

⁴Yeh, F.C., and D. M. O'Malley. Enzyme variations in natural populations of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) from British Columbia. Ms. submitted to *Silvae Genetica*.

⁵Personal communication from D. M. O'Malley, University of Wisconsin, Madison, July 1979.

Table 3—Mean genetic distance estimates, D , for a number of species and sampling units with estimates arranged in order of increasing $\bar{D}$

Species	Loci	$\bar{D}$	Sampling unit	Source
Douglas-fir	21	0.004	Coastal British Columbia	Yeh and O'Malley (in preparation)
Pitch pine	21	.007	Eastern United States	Guries and Ledig (in preparation)
Silver spot butterfly	3	.013	Northern California	Brittnacher and others 1978
Norway spruce	4	.014	Sweden	Lundkvist and Rudin 1977
House mouse	41	.014	Single barn	Selander, Hunt and Young 1969 (see Nei 1972)
Phlox	20	.019	Central Texas	Levin 1978
Mosquitos	18	.042	Coastal Kenya	Tabachnick and others 1979
American Indian tribes	6	.053	Northern South America	Ward and others 1975
Topminnow	25	.119	Northwestern Mexico	Vrijenhoek and others 1977
Pocket gophers	23	.144	Southwestern United States	Patton and Yang 1977
Newts	35 to 40	.292	River drainage in California	Hedgecock 1978

Table 4—Product-moment correlations between frequency of most common allele for 11 loci in pitch pine and selected climatic variables

Climatic variable ¹	Locus ²										
	MDH-1	MDH-2	IDH	6-PG-1	6-PG-2	G-6-P	LAP-1	LAP-2	GOT-1	GOT-2	ACO
Min. Jan. temp.	-0.06	0.406	0.655*	-0.718**	-0.367	-0.743**	-0.12	0.275	-0.776**	0.131	-0.502
Max. July temp.	.514	-.19	.432	-.342	.175	-.002	.139	.675	.036	-.257	.228
Heating ° days	-.18	-.577*	-.664*	.693*	.272	.734**	.095	-.477	.693*	.333	.333
Annual precipitation	-.042	.557	.453	-.401	-.463	-.772**	-.743**	-.181	-.514	-.344	-.441
Total snowfall	-.150	-.588*	.440	.687*	.338	.779**	.191	-.279	.721**	-.048	.292

¹ Source: 30-year climatic data summaries from NOAA Environmental Data Service (1979).

² *Statistically significant at 5 percent level.

**Statistically significant at 1 percent level.

of phenotypic variation noted in wood specific gravity (Ledig and others 1975) and cone serotiny (Ledig and Fryer 1972), and genetic variation in height growth revealed in a common garden study (Ledig and others 1976). In all of these studies patterns of variation were well correlated with ecological variables considered important to growth or survival. In the present study, for four of six loci, significant correlations between allele frequencies and climatic variables involved winter temperature or snowfall, which perhaps, reflect environmental stress; conditions which tend to reduce survival and limit reproductive rate in pitch pine.

DISCUSSION

The overall picture of pitch pine emerging from these analyses is one of a weakly differentiated series of populations. Although isolation by distance appears to be an important aspect of differentiation in herbaceous plant species, it is only mildly so in pitch pine. The lack of significant barriers to gene flow may be one important reason for the relatively undifferentiated status of pitch pine with regard to allozyme loci. Although the range of pitch pine includes disjunct populations, no major geographic barriers to gene flow exist, and the time since pitch pine migrated from its glacial refugium to occupy its present range has been brief, less than 100 to 300 generations. Furthermore, pitch pine, like most north-temperate tree species, is anemophilous, and wind can be an effective pollen vector for long distance transport (Koski 1970). In addition, pitch pine shows a dramatic depression when subjected to inbreeding (Wright 1962); therefore, selection reduces the impact of inbreeding and favors a strong tendency to outbreeding. For genetic distance, the estimates for pitch pine and other tree species (table 3) are similar and smaller than similar comparisons with inbreeding herbaceous species. The characteristic long generations and open breeding systems shared by tree species may act to distinguish them from herbaceous species.

Nevertheless, the data are insufficient to determine whether the results observed for pitch pine are really typical of other forest trees. Our current opinion is that

they will prove to be reasonably typical of many other eastern conifers. Similarities in breeding system, demography, range, time since post-Pleistocene recolonization, and other factors all suggest that patterns paralleling that of pitch pine might be expected. Conifers such as eastern hemlock (*Tsuga canadensis* [L.] Carr.), which is a late successional species, red pine (*Pinus resinosa* Ait.), which has limited genetic variability (Fowler and Morris 1977), or jack pine (*Pinus banksiana* Lamb.) with an extensive range, may differ from the pitch pine model. Conifers of the Western United States may be relatively more differentiated than pitch pine, especially those that have extensive and fractionated populations in mountainous areas where opportunities for differential selection are great. In mountainous areas population structure in its spatial aspects conforms to an island model, and is conducive to differentiation among populations. Some hardwoods, those that are insect-pollinated, may also deviate considerably from the pitch pine model, but most anemophilous hardwoods should parallel pitch pine. Electrophoretic studies of these and other species will undoubtedly increase our knowledge of the genetic structure and differentiation characteristic of forest trees. Results from such studies should be of great interest and value to population biologists and tree breeders alike.

Acknowledgments

This study was supported by Grant DEB74-11794, from the U.S. National Science Foundation and Regional Research Project 142-1826 from the Science and Education Administration, U.S. Department of Agriculture, and by the College of Agriculture and Life Sciences, University of Wisconsin, Madison.

LITERATURE CITED

- Avise, J. C.
1974. Systematic value of electrophoretic data. *Syst. Zool.* 23:465-481.
Avise, J. C., and J. Felley.
1979. Population structure of freshwater fishes. I. Genetic variation of bluegill (*Lepomis macrochirus*) populations in man-made reservoirs. *Evolution* 33:15-26.
Brittnacher, J. G., S. R. Sims, and F. J. Ayala.
1978. Genetic differentiation between species of the genus *Speyeria* (Lepidoptera: Nymphalidae). *Evolution* 32:199-210.

- Brown, A. H. D.
1978. **Isozymes, plant population genetic structure and genetic conservation.** Theor. Appl. Genet. 52:145-157.
- Eanes, W. F., and R. K. Koehn.
1978. **An analysis of genetic structure in the Monarch butterfly, *Danaus plexippus*.** Evolution 32:784-797.
- Feret, P. P.
1974. **Genetic differences among three small stands of *Pinus pungens*.** Theor. Appl. Genet. 44:173-177.
- Fowler, D. P., and R. W. Morris.
1977. **Genetic diversity in red pine: evidence for low genic heterozygosity.** Can. J. For. Res. 7:343-347.
- Fryer, J. H., and F. T. Ledig.
1972. **Microevolution of the photosynthetic temperature optimum in relation to the elevation] complex gradient.** Can. J. Bot. 50: 1231-1235.
- Gottlieb, L. D.
1977. **Electrophoretic evidence and plant systematics.** Ann. Missouri Bot. Gard. 64:161-180.
- Hedgecock, D.
1978. **Population subdivision and genetic divergence in red-bellied newt, *Taricha rivularis*.** Evolution 32:271-286.
- Jain, S. K.
1975. **Population structure and the effects of breeding system.** In Crop genetic resources for today and tomorrow. O. H. Frankel and J. G. Hawkes, eds., p. 15-36. Cambridge Univ. Press, Cambridge.
- Kirby, G. C.
1975. **Heterozygote frequencies in small subpopulations.** Theor. Popul. Biol. 8:31-48.
- Koski, V.
1970. **A study of pollen dispersal as a mechanism of gene flow in conifers.** Communicationes Instituti Forestalis Fenniae 70.4. Helsinki, Finland.
- Ledig, F. T., and J. H. Fryer.
1972. **A pocket of variability in *Pinus rigida* Mill.** Evolution 26: 259-266.
- Ledig, F. T., C. C. Lambeth, and D. I. H. Linzer.
1976. **Nursery evaluation of a pitch pine provenance trial.** In Proc. 23d Northeast. Forest Tree Improv. Conf., New Brunswick, N. J. p. 93-108.
- Ledig, F. T., B. J. Zobel, and M. F. Matthias.
1975. **Geoclimatic patterns in specific gravity and tracheid length in wood of pitch pine.** Can. J. For. Res. 5:318-329.
- Levin, D. A.
1978. **Genetic variation in annual phlox: self-compatible versus self-incompatible species.** Evolution 32:245-263.
- Lewontin, R. C.
1974. **The genetic basis of evolutionary change.** 346 p. Columbia Univ. Press, N. Y.
- Lundkvist, R., and D. Rudin.
1977. **Genetic variation in eleven populations of *Picea abies* as determined by isozyme analysis.** Hereditas 85:67-74.
- Mitton, J. B., Y. B. Linhart, J. L. Hamrick, and J. S. Beckman.
1977. **Observations on the genetic structure and mating system of ponderosa pine in the Colorado front range.** Theor. Appl. Genet. 51:5-13.
- Neale, D.
1978. **Allozyme studies in balsam fir.** M.S. thesis. Univ. New Hampshire, Durham.
- Neel, J. V., and R. J. Ward.
1972. **The genetic structure of a tribal population, the Yanomama Indians. VI. Analysis of F-statistics (including a comparison with the Makiritare and Xavante).** Genetics 72:639-666.
- Nei, M.
1965. **Variation and covariation of gene frequencies in subdivided populations.** Evolution 19:256-258.
- Nei, M.
1972. **Genetic distance between populations.** Amer. Nat. 106:283-292.
- Nei, M.
1975. **Molecular population genetics and evolution.** 288 p. Amer. Elsevier Publ., N. Y.
- Nei, M.
1977. **F-statistics and analysis of gene diversity in subdivided populations.** Ann. Human Genet. 41:225-233.
- Nei, M., and Y. Imaizumi.
1966. **Genetic structure of human populations. I. Local differentiation of blood group gene frequencies in Japan.** Heredity 21:9-35.
- NOAA-Environmental Data Service
1978. **Climatological data. National summary.** V. 27 (13) annual summary. National Climate Center, Asheville, N.C. 128 p.
- Patton, J. L., and S. Y. Yang.
1977. **Genetic variation in *Thomomys bottae* pocket gophers: macrogeographic patterns.** Evolution 31:697-720.
- Prakash, S.
1973. **Patterns of gene variation in central and marginal populations of *Drosophila robusta*.** Genetics 75:347-369.
- Schaal, B.
1974. **Isolation by distance in *Liatris cylindracea*.** Nature 252:703.
- Schaal, B.
1975. **Population structure and local differentiation in *Liatris cylindracea*.** Amer. Nat. 109:511-528.
- Selander, R. K.
1970. **Behavior and genetic variation in natural populations.** Amer. Zool. 10:53-66.
- Selander, R. K., and D. W. Kaufman.
1975. **Genetic structure of populations of the brown snail (*Helix aspersa*). I. Microgeographic variation.** Evolution 29:385-401.
- Selander, R. K., W. G. Hunt, and S. Y. Yang.
1969. **Protein polymorphism and genetic heterozygosity in two European subspecies of the house mouse.** Evolution 23:279-290.
- Snedecor, G., and M. R. Irwin.
1933. **On the chi-square test for homogeneity.** Iowa State Coll. J. Sci. 8:75-81.
- Tabachnick, W. J., L. E. Munstermann, and J. R. Powell.
1979. **Genetic distinctness of sympatric forms of *Aedes aegypti* in East Africa.** Evolution 33:287-295.
- Vrijenhoek, R. C., R. A. Angus, and R. J. Schultz.
1977. **Variation and heterozygosity in sexually versus clonally reproducing populations of *Poecidopsis*.** Evolution 31:767-781.
- Ward, R. H., H. Gershowitz, M. Layrisse, and J. V. Neel.
1975. **The genetic structure of a tribal population, the Yanomama Indians. XI. Gene frequencies for 10 blood groups and the ABH-Le secretor traits in the Yanomama and their neighbors; the uniqueness of the tribe.** Amer. J. Human Genet. 27:1-30.
- Workman, P. L.
1969. **The analysis of simple genetic polymorphisms.** Human Biol. 41: 97-114.
- Workman, P. L. and J. D. Niswander.
1970. **Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago.** Amer. J. Human Genet. 22: 24-49.
- Wright, J. W.
1962. **Genetics of forest tree improvement.** 399 p. FAO, Rome.
- Wright, J. W.
1976. **An introduction to forest genetics.** 463 p. Academic Press, N.Y.
- Wright, S.
1951. **The genetical structure of populations.** Ann. Eugen. 15:323-354.
- Wright, S.
1965. **The interpretation of population structure by F-statistics with special regard to systems of mating.** Evolution 19:395-420.
- Wright, S.
1969. **Evolution and the genetics of populations. V. II. The theory of gene frequencies.** 511 p. Univ. Chicago Press, Chicago.