STRUCTURE OF GENETIC VARIATION WITHIN AND BETWEEN POPULATIONS OF MYCOPHAGOUS DROSOPHILA

ROBERT C. LACY[1]

Section of Ecology and Systematics, Cornell University, Ithaca, New York 14853

Manuscript received July 17, 1982
Revised copy accepted January 18, 1983

ABSTRACT

Patterns of genetic variation within and between populations of five species of mycophagous Drosophila were examined by gel electrophoresis of several polymorphic loci. Populations of the five species could not be shown to be subdivided into sympatric host-adapted races. Statistically significant, but small, between-host differences in gene frequencies were observed at three of 15 loci. Mean gene frequencies at all loci were similar in New York and Tennessee, and, with one exception, relatively little genetic differentiation was observed among study sites within those two regions. Gene frequencies generally were stable over several years of collecting as well. The unpredictable nature of the fungal hosts may preclude the site fidelity and continuity of diversifying selection necessary for adaptive divergence of populations.

THE evolutionary importance of sympatric formation of host-specific racial differences, leading at times to full biological species, was proposed early (WALSH 1864) but was then generally discredited as lacking both a plausible theoretical basis and supporting data (MAYR 1947, 1963). Recently, however, it has become the subject of renewed interest (e.g., HEED 1971), support (e.g., BUSH 1975; WHITE 1978) and rebuttal (e.g., FUTUYMA and MAYER 1980; JAENIKE 1981). MAYNARD SMITH (1966) proposed a rigorous model by which sympatric speciation could occur. Stating that "the crucial first step in sympatric speciation is the establishment of a stable polymorphism in a heterogeneous environment," he first showed (as had LEVENE 1953) that such multiple niche polymorphisms could be maintained in a randomly mating population if the selective advantages of different genotypes in different habitats are quite large, or if they are delicately adjusted to the relative carrying capacities of each habitat. Sympatric speciation could follow if genotypes showed a preference for the habitat in which they were raised. Recently, claims or suggestions have been made of sympatric speciation within natural populations of tephritid fruit flies (BUSH 1969, 1974), diprionid sawflies (KNERER and ATWOOD 1973), lacewings (TAUBER and TAUBER 1977), goodeid fish (TURNER and GROSSE 1980) and membracid treehoppers (WOOD 1980; GUTTMAN, WOOD and KARLIN 1981; WOOD and GUTTMAN 1982). It has also been suggested that some Hawaiian Drosophila (RICHARDSON 1974) or the *Drosophila melanogaster-simulans* sibling species pair

[1] Present address: Department of Biology, Franklin and Marshall College, Lancaster, Pennsylvania 17604.

(PARSONS 1981) could have diverged under sympatry. As pointed out by FUTUYMA and MAYER (1980), however, most if not all of the purported cases of sympatric speciation in animals still lack evidence either that divergence occurred sympatrically or that genetic divergence has progressed to the extent that continued gene flow does not occur. Thus, there is need for further evidence that multiple niche polymorphism can lead first to host race formation and then to speciation without the external barriers to gene flow once thought to be prerequisite.

The mushroom-feeding Drosophila (Drosophilidae: Diptera) fit many of the suggested conditions for promoting host race formation. Individual larvae are restricted to a single mushroom cap. Populations of generalist species (those utilizing many species of host mushrooms) have more genic variation than do the more specialized species, suggesting that between-host selective differences maintain multiple niche polymorphisms (LACY 1982a). Hosts are probably located by chemical cues and mating occurs directly on the hosts [traits that favor genetic and conditioned host selection (THORPE 1945; BUSH 1969)]. Finally, hosts are often quite distinct chemically, morphologically and with respect to spatial and temporal distribution.

Fungal hosts are often ephemeral, however, while stable resources may be requisite for adaptive genetic divergence (JAENIKE and SELANDER 1979; GUTTMAN, WOOD and KARLIN 1981). These mycophagous Drosophila breed continuously from May through October, but many of their host species fruit primarily during just a few of those months. Only one (*Polyporus squamosus*) was found fruiting continuously throughout the fly breeding season. Although individual mushroom species are rarely reliable hosts, higher taxa of fungi may offer suitable resources for specialization. Representatives of the Clavariaceae, Russulaceae, Amanitaceae, and Boletaceae were easy to locate throughout my collecting in Tennessee. Drosophilid species are differentially attracted to these mushroom families (LACY 1983), and it is possible that populations within these species also have evolved host preferences.

JAENIKE (1978) did not find individual differences in host preferences among *D. falleni*, *D. putrida* and *D. testacea* that he marked on three species of Amanita and one species of Russula; *D. falleni* collected from seven species of Amanita were not genetically differentiated (JAENIKE and SELANDER 1979). To test further for genetic differentiation among conspecific populations of mycophagous Drosophila, I examined the amount of genetic divergence among populations of five species of mycophagous Drosophila that were separated spatially, temporally and with respect to host use.

MATERIALS AND METHODS

Drosophilid flies were aspirated as adults or reared from mushrooms found in study sites in Tompkins County, New York, and the Great Smoky Mountains National Park, Tennessee. The New York sites were sampled from June to October, 1978; in June and September, 1979 and 1980; and in June, August, and September, 1981. The Smoky Mountain sites were sampled in July and August, 1979–1981. During approximately weekly visits to each site, the area (about 60 × 60 m) was searched for 1–3 hr during the morning (usually 7 to 10 am). An attempt was made to locate any mushrooms, to collect all drosophilid flies on the mushrooms and to collect a representative sample of mushrooms

from which flies would be reared in the laboratory. Detailed site descriptions and collection data are given in LACY (1982b). Preliminary genetic analyses indicated that flies reared from a mushroom often consist of the progeny of one or a few females and, thus, would not provide independent random samples of the gene pool. Insufficient numbers of flies were reared from most mushroom caps to permit the analysis of variation between individual mushroom caps, within host species, that would be required to factor out the between-sibship component of between-host variation. Adult flies in an area are more likely a mixture of many progenies, and, therefore, only the flies collected as adults were used in analyses of genetic differentiation. Gene frequency differences caused by selection on larval populations inhabiting different hosts may be obscured if the mobile adults do not tend to return to their larval hosts for mating, ovipositing and feeding; but such a lack of host conditioning or habitat preference would also preclude host-adapted differentiation. Adequate samples of five fly species (*D. falleni*, *D. putrida*, *D. testacea*, *D. tripunctata* and *D. ordinaria*) remained after a survey of genetic variability (LACY 1982a) to allow characterization of levels of genetic differentiation among populations sampled from mushroom species in various genera and families, collected during several months and years, and from sites in both New York and Tennessee.

Genetic variation was assessed by horizontal starch gel electrophoresis, as described in LACY (1982a,b). For each fly species, a set of loci was chosen all of which were polymorphic, could be run on the same gel-electrode buffer system and usually produced easily scored patterns of activity on stained gels. The loci, given in Table 1, were run on a Tris-citrate buffer system (electrode buffer: 0.75 M Tris, 0.25 M citric acid, pH 6.9; gel buffer: 1:60 dilution of electrode buffer). Large numbers of flies were assayed for the chosen loci, with an attempt being made to test samples from different study sites, months and host mushrooms in a scrambled order to prevent possible bias introduced by electrophoretic techniques. Flies from New York and Tennessee were frequently run on the same gels, assuring identical scoring of electromorphs in flies from the two regions.

For each locus, genetic variation within and between samples was measured by the expected heterozygosity. *F*-statistics (WRIGHT 1951, 1965, 1978; NEI 1977) were used to quantify the proportion (F_{RT}) of the total heterozygosity due to gene frequency differences between geographic regions (Tompkins County, New York, and the Great Smoky Mountains, Tennessee), the proportion of the within-region variation due to differences between study sites (F_{SR}), the proportion of within-site variation due to gene frequency changes between years (F_{YS}), the proportion of within-year variation due to gene frequency changes between months (F_{MY}), the proportion of within-month variation due to gene frequency differences between flies collected from different host mushroom species (F_{HM}) and, the remainder, the proportion of the total heterozygosity represented within local host populations ($1 - F_{HT}$). An indication as to whether any further population substructuring exists is given by the magnitude of the deviation (F_{IH}) of observed heterozygosities within each local host population from the expected heterozygosities.

In addition to these descriptive statistics, I tested for significant heterogeneities in gene frequencies with *G*-statistics based on log likelihood ratios (FIENBERG 1977; SOKAL and ROHLF 1981). At each hierarchical level, the *G*-test compared the electromorph frequencies observed when populations are partitioned down to that level with the frequencies observed with one fewer levels of classification (*i.e.*, the values expected if no differences exist at the level under consideration). Deviations from Hardy-Weinberg equilibrium (F_{IH}) were tested by use of a *G*-test comparing expected and observed heterozygosities within each local host population. *F*-values were not tested directly for significance. In some cases flies were available from only one class within the next higher level of the hierarchy (e.g., flies collected from only one mushroom species within a month), and so the expected heterozygosity for that sample would be the same at both hierarchical levels. To avoid bias, each *F*-statistic was calculated from data that excluded such cases. Degrees of freedom in *G*-tests were also adjusted to account for expected and observed values that were equal due to the structure of the data.

My hierarchical analysis of the data imposes a stringent test for host-adapted differentiation. Allozyme differences over spatial and temporal scales may result in part from adaptive responses to different mushroom associations. Conversely, genetic divergence between spatially and temporally diverse populations collected from different host species may have adaptive and nonadaptive causes only spuriously correlated with host utilization. By first factoring out geographic variation, I chose to include all causes of spatial differences in F_{RT} and F_{SR}. Similarly, my measures of temporal variation (F_{YS} and F_{MY}) incorporate any genetic responses to selection that varies because

TABLE 1

Partitioning of genetic variability within species of mycophagous Drosophila into spatial, temporal and between-host components

Species locus	N	H_T	F_{RT}	F_{SR}	F_{YS}	F_{MY}	F_{HM}	$1 - F_{HT}$	F_{IH}
falleni									
Acp	652	0.444	0.003*	0.008	0.003	0.003	0.023	0.968	0.002
IDH	1266	0.028	0.001	0.003	0.002	0.003	0.002	0.994	−0.018
PGM	4028	0.604	0.010***	0.002	0.003*	0.005**	0.013	0.972	0.049
putrida									
Acp	758	0.406	0.014***	0.031***	0.175***	0.218***	0.016	0.768	−0.015
PGI	1832	0.315	0.000	0.009**	0.005	0.010	0.013	0.971	−0.009
PGM	1862	0.547	0.000	0.002	0.002	0.004	0.012	0.986	0.007
testacea									
IDH	786	0.486	0.018***	0.033***	0.020	0.057**	0.007	0.928	−0.027
PGI	758	0.473	0.014**	0.018*	0.002	0.026	0.018*	0.951	−0.097
PGM	830	0.532	0.002**	0.012	0.021	0.008	0.008	0.975	−0.017
tripunctata									
IDH	786	0.035	0.000	0.012	0.003	0.005	0.057	0.940	−0.078
LAP	510	0.203	0.003	0.022		0.003	0.013	0.965	−0.020
MDH2	1004	0.117	0.000	0.358***	0.038	0.032	0.027*	0.606	−0.127
PGM	1124	0.443	0.002	0.006	0.003	0.008	0.015	0.973	−0.012
ordinaria									
Acp	672	0.322	0.002	0.033***	0.001		0.032**	0.943	0.227
PGM	1198	0.326	0.001	0.002	0.002		0.006	0.991	0.033
Means			0.005	0.037	0.020	0.029	0.017	0.927	−0.007

Columns of numbers are: total numbers of alleles scored (N), total diversity of electromorphs (H_T) as measured by the expected heterozygosities, and a hierarchical partitioning with F-statistics into between-region (F_{RT}), between-site (F_{SR}), between-year (F_{YS}), between-month (F_{MY}), between host (F_{HM}), within-host ($1 - F_{HT}$) and between-individual (F_{IH}) components of heterozygosity. Asterisks indicate statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) of the corresponding G-tests for heterogeneity of electromorph frequencies. *Acp* = acid phosphatase; *IDH* = isocitrate dehydrogenase; *LAP* = leucine amino peptidase; *MDH2* = malate dehydrogenase (anodal); *PGI* = phosphoglucose isomerase; *PGM* = phosphoglucomutase.

of changing host availability over time. Between-host variation (F_{HM}) was narrowly defined as divergence observed between populations of flies collected from various species of mushrooms, within small study sites, within years and within months. This conservative test cannot rule out the possibility of adaptive between-host divergence over allopatric and allochronic scales, yet only a finding of host race differentiation in synchronous samples of local populations would provide strong evidence for the sympatric divergence of populations. Finally, there is little overlap in the mushroom hosts commonly found in New York and Tennessee, and the two regions were necessarily sampled at different times. Thus, it would not be possible statistically to remove host-adaptation and temporal gene frequency change from measures of geographic variation.

RESULTS

The partitioning of genetic diversity into spatial, temporal and between-host components is given in Table 1. (Electromorph frequencies summarized by site,

month and host species are given in LACY 1982b.) In many cases gene frequencies were not found to differ significantly between pooled New York and pooled Tennessee samples. Diversity components lower in the hierarchy were generally greater than the between-region values, although less often statistically significant. At lower levels sample sizes necessarily diminish, and so the sampling errors in estimating gene frequencies increase. This both artificially elevates measures of between subpopulation differentiation and increases the amount of genetic differentiation necessary for statistical significance. Furthermore, if

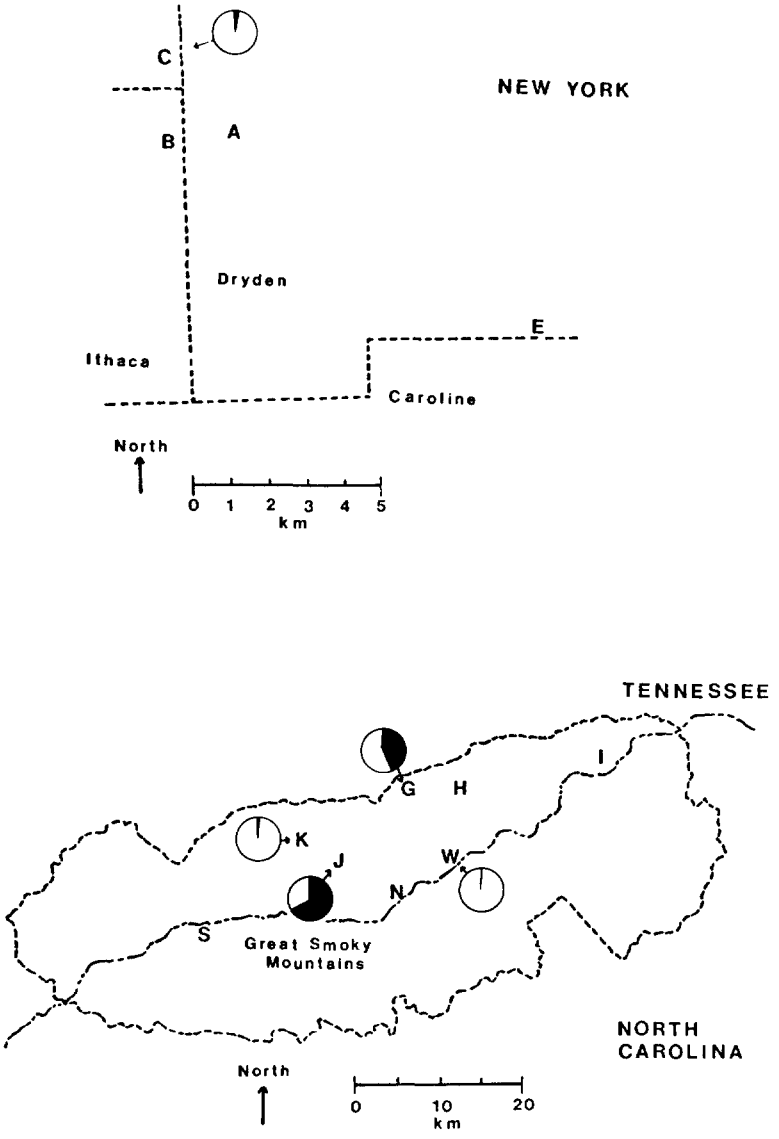

FIGURE 1.—Electromorph frequencies at the *MDH2* locus observed in populations of *D. tripunctata*. Open areas = allele 1.00; solid areas = allele 2.00 and rare variants. Sample sizes: site C, 24 alleles scored; G, 32; J, 38; K, 386; W, 516.

broader scales of sampling result in pooling data across many Mendelian populations, a patchwork of locally differentiated demes would be blended into mean regional frequencies and, thus, obscured. The patterns of spatial differentiation are shown in Figures 1-6 for those loci at which significant between-site differences were observed. (Frequencies are given for all sites at which sample sizes exceeded 20 alleles scored.) The electromorph frequencies show no relationship either to geographic distance between localities or to obvious

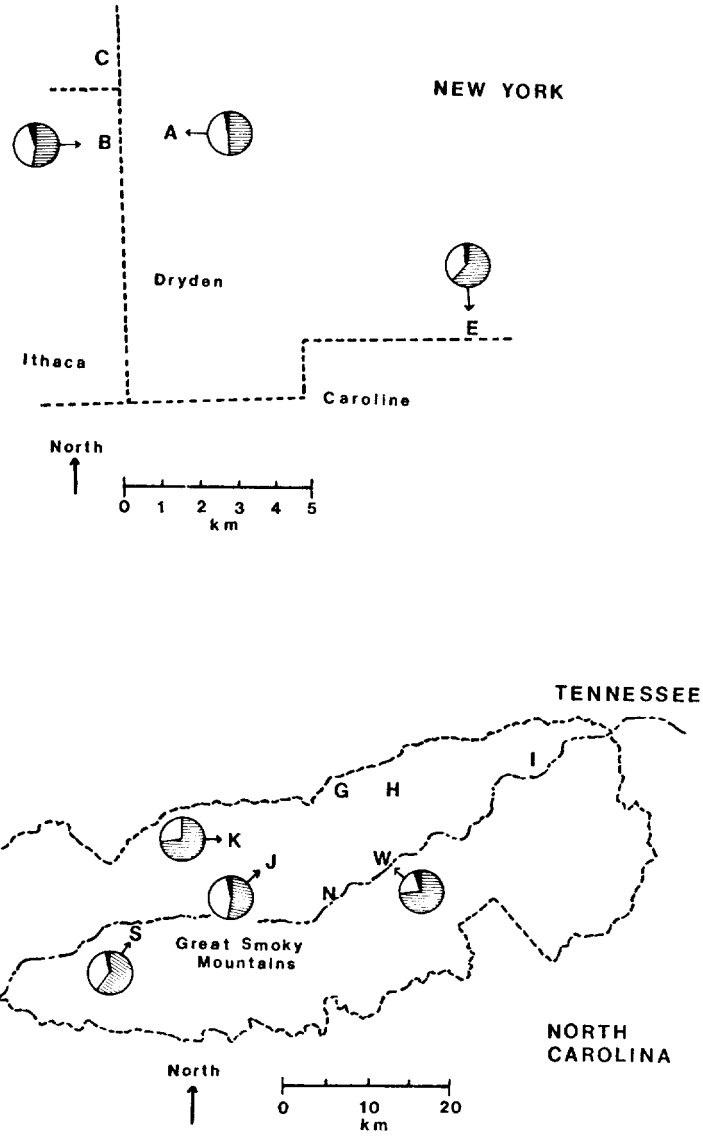

FIGURE 2.—Electromorph frequencies at the *IDH* locus of *D. testacea*. Hatched = allele 1.00; open = allele 1.20; solid = minor alleles. Sample sizes: site A, 64; B, 76; E, 38; J, 128; K, 60; S, 54; W, 350 alleles scored.

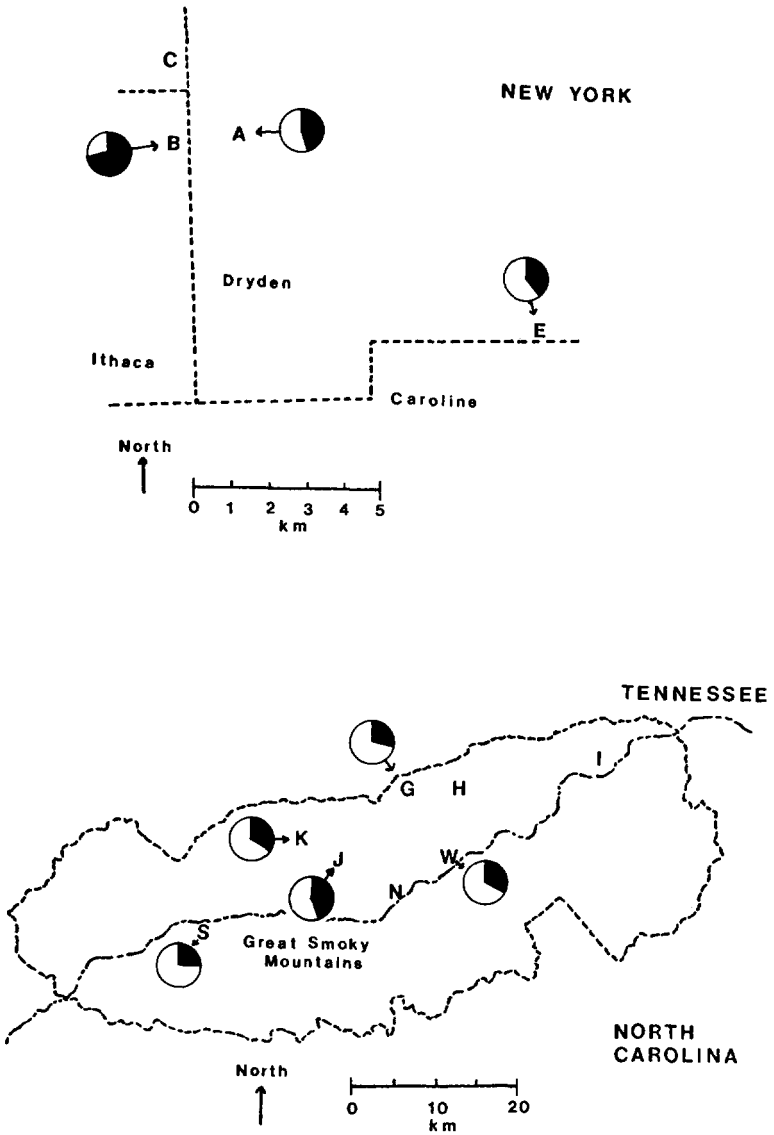

FIGURE 3.—Electromorph frequencies at the *PGI* locus of *D. testacea*. Solid = allele 0.69; open = allele 1.00 and minor alleles. Sample sizes: site A, 64; B, 28; E, 28; G, 28; J, 136; K, 60; S, 44; W, 362 alleles scored.

ecological differences. For example, at the one locus showing strong spatial differentiation, an *MDH-2* allele in *D. tripunctata* is common in my sites G and J in the Smoky Mountains and very rare in sites K and W (Figure 1); yet, G and K are low elevation sites with similar mushroom floras (LACY 1982b), J is a mid-elevation site about 5 km upriver from K and W is a high elevation site closest to site G. With respect to the other loci, the fly populations seem generally to consist of spatially and ecologically random patterns of slightly differentiated local demes.

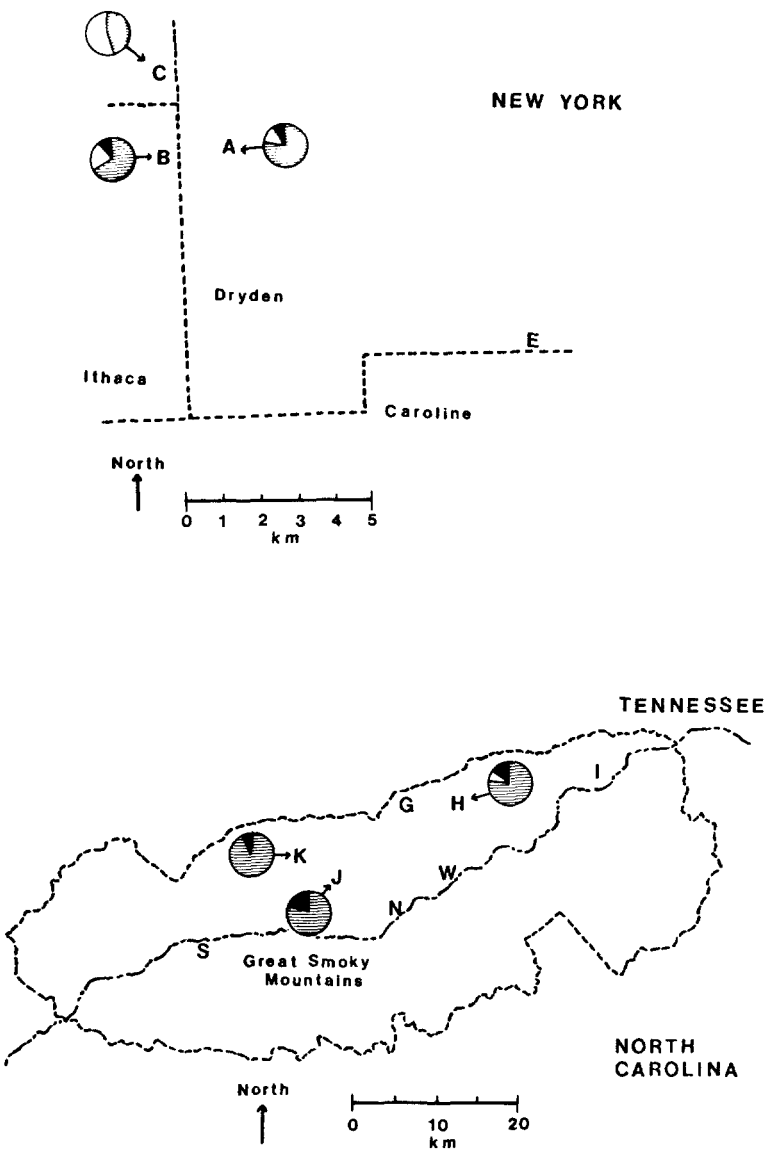

FIGURE 4.—Electromorph frequencies at the *Acp* locus of *D. putrida*. Hatched = allele 0.09; open = allele 0.70; solid = other alleles. Sample sizes: site A, 322; B, 220; C, 22; H, 82; J, 66; K, 40 alleles scored.

At only one locus (*D. putrida*, *Acp*) was marked temporal differentiation observed. No clear seasonal cycles or directional trends were apparent at this locus; rather, frequencies seem to vary randomly over time.

Statistically significant between-host genetic divergence was observed at the *PGI* locus of *D. testacea* ($P < 0.05$), the *MDH-2* locus of *D. tripunctata* ($P < 0.05$) and the *Acp* locus of *D. ordinaria* ($P < 0.01$), although only a few percent of the

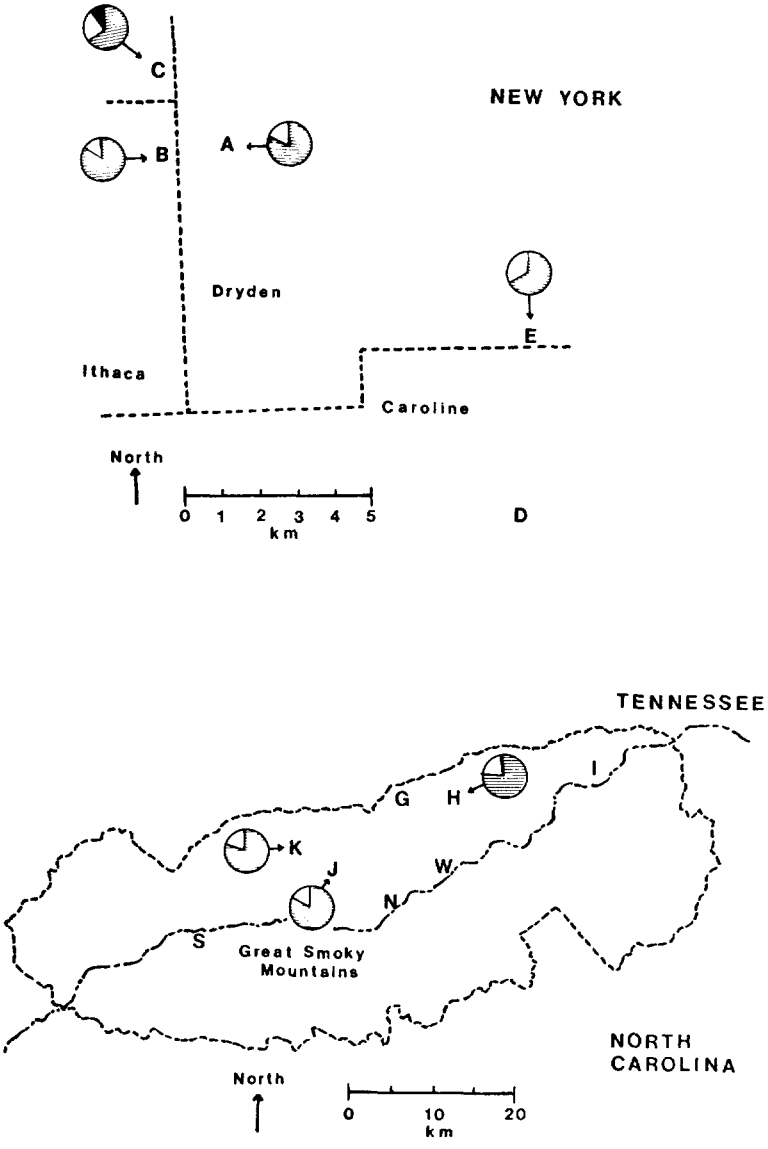

FIGURE 5.—Electromorph frequencies at the *PGI* locus of *D. putrida*. Hatched = allele 1.00; open = allele 0.75; solid = minor alleles. Sample sizes: site A, 380; B, 276; C, 50; E, 32; H, 160; J, 398; K, 522 alleles scored.

heterozygosity was due to gene frequency differences between flies collected from different host mushroom species. In general about 90% of the total heterozygosity was represented within local host populations ($1 - F_{HT}$). The observed heterozygosities within these host populations conformed to Hardy-Weinberg expectations. In many cases there was a slight excess of heterozygotes, but deviations were never statistically significant.

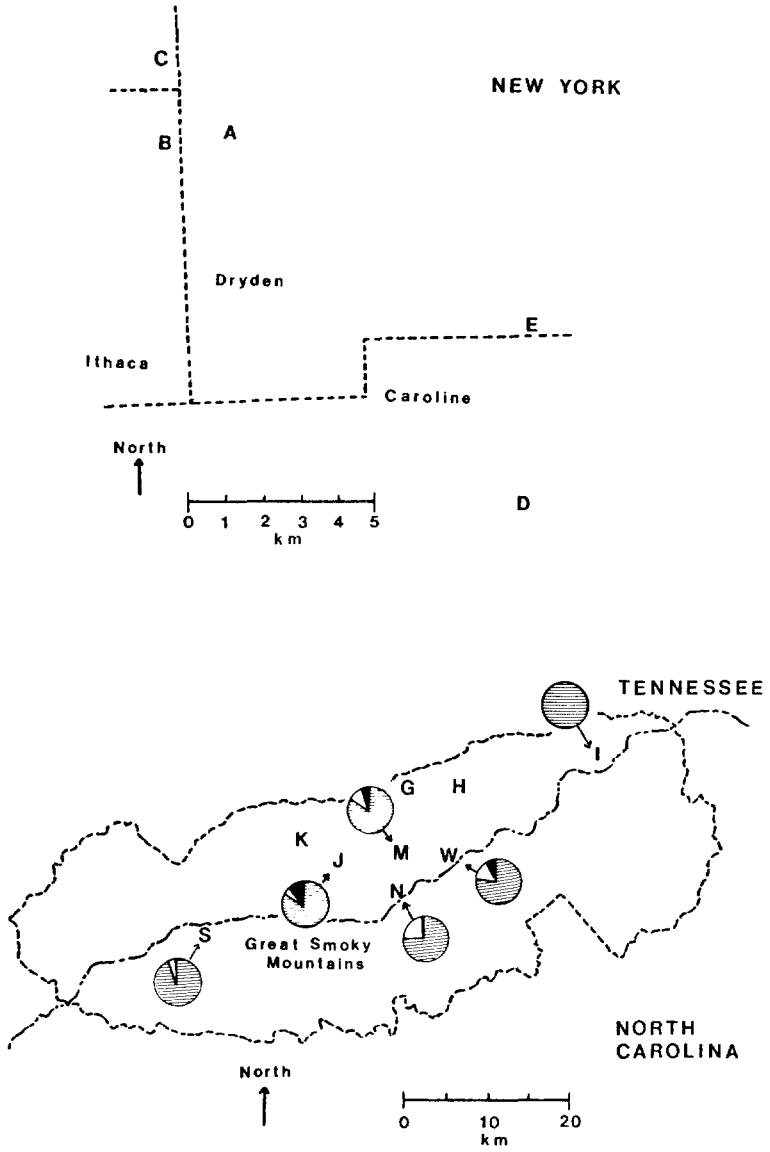

FIGURE 6.—Electromorph frequencies at the *Acp* locus of *D. ordinaria*. Hatched = allele 1.00; open = allele 3.00; solid = minor alleles. Sample sizes: site I, 24; J, 20; M, 52; N, 224; S, 82; W, 260 alleles scored.

DISCUSSION

At several loci electromorph frequencies varied more among host mushrooms than expected by chance; yet, the differentiation was slight. Between-host differences in gene frequencies could result either from natural selection favoring different genetic alleles on different hosts or from differential host preferences by genotypes. Since 15 loci were examined, however, one or more of these statistically significant results may have been spurious.

Although other loci, especially any that code for larval-specific enzymes, may show greater between-host divergence, populations of the five species studied are not noticeably differentiated into host races and do not seem to be proceeding toward sympatric speciation. Either gene flow prevents adaptive divergence [as concluded by JAENIKE and SELANDER (1979), in their study of *D. falleni*] or there exist little selective difference between the host mushroom species for those loci examined, or both. Although correlations of mean heterozygosities (averaged over 12 to 30 loci) with niche breadths suggest that selection acts on at least some electromorphs (LACY 1982a), there is no assurance that the few polymorphic loci chosen for this study were under diversifying selection.

Examination of patterns of differentiation at higher levels may provide insight into the population structures of these fly species and perhaps may suggest likely causes for the lack of observed differentiation at the level of host populations. With the exception of the *MDH-2* locus in *D. tripunctata*, there was little spatial structure evident in the electromorph frequencies. Studies of other Drosophila species also have found fairly uniform gene frequencies across ranges similar in scale to my New York-Tennessee comparisons (PRAKASH, LEWONTIN and HUBBY 1969; PRAKASH 1973; ROCKWOOD-SLUSS, JOHNSTON and HEED 1973). From these data sets and others, EANES and KOEHN (1978) calculated F_{ST} values of 0.030 for *D. pseudoobscura*, 0.044 for *D. melanogaster* and 0.055 for *D. robusta*. These are greater than the comparable values (F_{RT}) in Table 1, although my pooling of samples from multiple study sites within each region could lead to this result. The differentiation I observed over a more local scale (mean $F_{SR} = 0.037$) is of a magnitude similar to the values reported for other Drosophila.

Such uniform gene frequencies might indicate widespread gene flow, yet Drosophila populations have been found to show microspatial differentiation over distances of 10–500 m with respect to alcohol tolerances (MCKENZIE and PARSONS 1974), chromosome inversion frequencies (STALKER 1976; TAYLOR and POWELL 1977) and allozymes (TAYLOR and POWELL 1977; RICHMOND 1978). Mark-recapture studies indicate that Drosophila may travel as few as 5–10 m/day (WALLACE 1970; JOHNSTON and HEED 1975) or as much as 1–15 km/day (JOHNSTON and HEED 1976; COYNE *et al.* 1982), with mobility strongly correlated with the distance between traps or favorable natural substrates (JOHNSTON and HEED 1975). Using WRIGHT'S (1965) approximation of $F_{ST} = 1/(4Nm + 1)$ for differentiation of neutral variants in a subdivided population at equilibrium, migration could be estimated at 50 flies per generation (Nm) between the Smoky Mountains, Tennessee and Tompkins County, New York (mean $F_{RT} = 0.005$, Table 1), and seven flies per generation between study sites within each region (mean $F_{SR} = 0.037$). Although mushrooms can usually be found within 10–50 m in the habitats studied, occasional long-distance dispersal could account for the spatial homogeneity in gene frequencies. *D. ordinaria* (= *D. magnafumosa* = *D. melanderi*, LACY 1981) has not been reported south of New York in the eastern United States, except at high elevations in the Smokies, and so would have to traverse 1000 km or more of unsuitable habitat for gene flow to occur; yet, this species has almost identical gene frequencies in New York and Tennessee.

The patterns of gene differences between sites are not uniformly indicative

of partially subdivided populations in which migration prevents differentiation of neutral alleles. Strong genetic differentiation was found at the *MDH-2* locus in *D. tripunctata* between sites as close as 5 km (Figure 1). The *MDH-2* locus follows a pattern in the Smokies that would be expected of selectively neutral variants in a subdivided population with low gene flow (Nm estimated at 0.45 migrants per generation). Spatial heterogeneity at the *MDH-2* locus could have resulted from strong selection acting differentially among the study sites. Alternatively, the relatively constant gene frequencies at other loci in *D. tripunctata*, and the other four species as well, may have been maintained by global selection stabilizing the populations against the random effects of drift.

Temporal differences in electromorph frequencies were similar to the spatial pattern. With one exception (*D. putrida*, *Acp*), frequencies were stable between months and between years. Although seasonal and directional changes are well documented for chromosome inversion frequencies in Drosophila (DOBZHANSKY 1970), allozyme studies often show less temporal variation in enzyme polymorphisms (BERGER 1971; DOBZHANSKY and AYALA 1973; CAVENER and CLEGG 1981).

Each mycophagous drosophilid larva is forced to be a specialist on the host in which it finds itself; yet, the adult population may utilize a variety of ephemeral mushrooms. Unpredictable resources may prevent the host fidelity by breeding populations necessary for adaptive radiation into host-specific races (BUSH and DIEHL 1983). Notably, most of the purported cases of host race formation are insects that specialize on predictable, long-lived host plants. Some Drosophilidae specialize on the long-lasting polypore bracket fungi (LACY 1983) and are relatively depauperate in allozymic variation (LACY 1982a). Due to the temporal and spatial stability of their host fungi, these specialists may be more likely than the generalists to develop genetic differentiation among local or host-specific populations. Tests of whether the more specialized mycophagous Drosophilidae feeding on long-lasting bracket fungi have population structures more conducive to host-adapted divergence must await further studies.

I am grateful to the National Park Service and especially DR. GARY LARSON, for encouraging and facilitating my research in the Great Smoky Mountains. PETER BRUSSARD, ROSS MACINTYRE and two anonymous reviewers made valuable comments on earlier drafts of this paper. The research was supported by grant DEB-7922141 from the National Science Foundation, and a United States Department of Agriculture Hatch allocation to Cornell University (P. F. BRUSSARD). Publication was made possible by a grant from Franklin and Marshall College.

LITERATURE CITED

BERGER, E. M., 1971 A temporal survey of allelic variation in natural and laboratory populations of *Drosophila melanogaster*. Genetics **67**: 121–136.

BUSH, G. L., 1969 Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis*. Evolution **23**: 237–251.

BUSH, G. L., 1974 The mechanism of sympatric host race formation in the true fruit flies (Tephritidae). pp. 3–23. In: *Genetic Mechanisms of Speciation in Insects*, Edited by M. J. D. WHITE. D. Reidel Publishers, Boston.

BUSH, G. L., 1975 Modes of animal speciation. Annu. Rev. Ecol. Syst. **6**: 339–364.

BUSH, G. L. and S. R. DIEHL, 1983 Host shifts, genetic models of sympatric speciation, and the

origin of parasitic insect species. In: *Fifth International Symposium on Insect-Plant Relationships*, Edited by J. H. VISSER and A. K. MINKS. Centre Agri. Publ. and Documentation (PUDOC), Wageningen. In press.

CAVENER, D. R. and M. T. CLEGG, 1981 Temporal stability of allozyme frequencies in a natural population of *Drosophila melanogaster*. Genetics **98:** 613–623.

COYNE, J. A., I. A. BOUSSY, T. PROUT, S. H. BRYANT, J. S. JONES and J. A. MOORE, 1982 Long-distance migration of *Drosophila*. Am. Nat. **119:** 589–595.

DOBZHANSKY, TH., 1970 *Genetics of the Evolutionary Process*. Columbia University Press, New York.

DOBZHANSKY, TH. and F. J. AYALA, 1973 Temporal frequency changes of enzyme and chromosomal polymorphisms in natural populations of *Drosophila*. Proc. Natl. Acad. Sci. USA **70:** 680–683.

EANES, W. F. and R. K. KOEHN, 1978 An analysis of genetic structure in the monarch butterfly, *Danaus plexippus* L. Evolution **32:** 784–797.

FIENBERG, S. E., 1977 *The Analysis of Cross-Classified Categorical Data*. M.I.T. Press, Cambridge, Massachusetts.

FUTUYMA, D. J. and G. C. MAYER, 1980 Non-allopatric speciation in animals. Syst. Zool. **29:** 254–271.

GUTTMAN, S. I., T. K. WOOD and A. A. KARLIN, 1981 Genetic differentiation along host plant lines in the sympatric *Enchenopa binotata* Say complex (Homoptera: Membracidae). Evolution **35:** 205–217.

HEED, W. B., 1971 Host plant specificity and speciation in Hawaiian *Drosophila*. Taxon **20:** 115–121.

JAENIKE, J., 1978 Host selection by mycophagous *Drosophila*. Ecology **59:** 1286–1288.

JAENIKE, J., 1981 Criteria for ascertaining the existence of host races. Am. Nat. **117:** 830–834.

JAENIKE, J. and R. K. SELANDER, 1979 Ecological generalism in *Drosophila falleni*: genetic evidence. Evolution **33:** 741–748.

JOHNSTON, J. S. and W. B. HEED, 1975 Dispersal of *Drosophila*: the effect of baiting on the behavior and distribution of natural populations. Am. Nat. **109:** 207–216.

JOHNSTON, J. S. and W. B. HEED, 1976 Dispersal of desert-adapted *Drosophila*—Saguaro-breeding *D. nigrospiracula*. Am. Nat. **110:** 629–651.

KNERER, G. and C. E. ATWOOD, 1973 Diprionid sawflies: polymorphism and speciation. Science **179:** 1090–1099.

LACY, R. C., 1981 Taxonomic and distributional notes on some fungus-feeding North American *Drosophila* (Diptera, Drosophilidae). Entomol. News **92:** 59–63.

LACY, R. C., 1982a Niche breadth and abundance as determinants of genetic variation in populations of mycophagous drosophilid flies (Diptera: Drosophilidae). Evolution **36:** 1265–1275.

LACY, R. C., 1982b Population biology of Mycophagous Drosophilidae in the Eastern United States, Ph.D. Thesis. Cornell University, Ithaca, New York.

LACY, R. C., 1983 Ecological and genetic responses to mycophagy in Drosophilidae. In: *Fungus/Insect Relationships. Perspectives in Ecology and Evolution*, Edited by Q. WHEELER and M. BLACKWELL. Columbia University Press, New York. In press.

LEVENE, H., 1953 Genetic equilibrium when more than one ecological niche is available. Am. Nat. **87:** 331–333.

MAYNARD SMITH, J., 1966 Sympatric speciation. Am. Nat. **100:** 637–650.

MAYR, E., 1947 Ecological factors in speciation. Evolution **1:** 263–288.

MAYR, E., 1963 *Animal Species and Evolution*. Harvard University Press, Cambridge, Massachusetts.

McKENZIE, J. A. and P. A. PARSONS, 1974 Microdifferentiation in a natural population of *Drosophila melanogaster* to alcohol in the environment. Genetics **77:** 385–394.

NEI, M., 1977 F-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. **41:** 225–233.

PARSONS, P. A., 1981 Habitat selection and speciation in *Drosophila*. pp. 219–240. In: *Evolution and Speciation. Essays in Honor of M. J. D. White*, Edited by W. R. ATCHLEY and D. S. WOODRUFF. Cambridge University Press, Cambridge.

PRAKASH, S., 1973 Patterns of gene variation in central and marginal populations of *Drosophila robusta*. Genetics **75:** 347–369.

PRAKASH, S., R. C. LEWONTIN and J. L. HUBBY, 1969 A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal, and isolated populations of *Drosophila pseudoobscura*. Genetics **61:** 841–858.

RICHARDSON, R. H., 1974 Effects of dispersal, habitat selection and competition on a speciation pattern of *Drosophila* endemic to Hawaii. pp. 140–164. In: *Genetic Mechanisms of Speciation in Insects*, Edited by M. J. D. WHITE. D. Reidel Publishers, Boston.

RICHMOND, R. C., 1978 Microspatial genetic differentiation in natural populations of *Drosophila*. pp. 127–142. In: *Ecological Genetics: The Interface*, Edited by P. F. BRUSSARD. Springer, Heidelberg.

ROCKWOOD-SLUSS, E. S., J. S. JOHNSTON and W. B. HEED, 1973 Allozyme genotype-environment relationships. I. Variation in natural populations of *Drosophila pachea*. Genetics **73:** 135–146.

SOKAL, R. R. and F. J. ROHLF, 1981 *Biometry*, 2nd ed. W. H. Freeman, San Francisco.

STALKER, H. D., 1976 Chromosome studies in wild populations of *D. melanogaster*. Genetics **82:** 323–347.

TAUBER, C. A. and M. T. TAUBER, 1977 A genetic model for sympatric speciation through habitat diversification and seasonal isolation. Nature **268:** 702–705.

TAYLOR, C. E. and J. R. POWELL, 1977 Microgeographic differentiation of chromosomal and enzyme polymorphisms in *Drosophila persimilis*. Genetics **85:** 681–695.

THORPE, W. H., 1945 The evolutionary significance of habitat selection. J. Anim. Ecol. **14:** 67–70.

TURNER, B. J. and D. J. GROSSE, 1980 Trophic differentiation in *Ilyodon*, a genus of stream-dwelling goodeid fishes: speciation vs. ecological polymorphism. Evolution **34:** 259–270.

WALLACE, B., 1970 Observations on the microdispersion of *Drosophila melanogaster*. pp. 381–399. In: *Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky*, Edited by M. K. HECHT and W. C. STEERE. Appleton-Century-Crofts, New York.

WALSH, B. D., 1864 On phytophagic varieties and phytophagic species. Proc. Entomol. Soc. Phila. **3:** 403–430.

WHITE, M. J. D., 1978 *Modes of speciation*. W. H. Freeman, San Francisco.

WOOD, T. K., 1980 Divergence in the *Enchenopa binotata* Say complex (Homoptera: Membracidae) effected by host plant adaptation. Evolution **34:** 147–160.

WOOD, T. K. and S. I. GUTTMAN, 1982 Ecological and behavioral basis for reproductive isolation in the sympatric *Enchenopa binotata* complex (Homoptera: Membracidae). Evolution **36:** 233–242.

WRIGHT, S., 1951 The genetical structure of populations. Ann. Eugen. (Lond.) **15:** 323–354.

WRIGHT, S., 1965 The interpretation of population structure by F-statistics with special regard to mating systems. Evolution **19:** 395–420.

WRIGHT, S., 1978 *Evolution and the Genetics of Populations*, Vol. **4**. Variability Within and Among Natural Populations. University of Chicago Press, Chicago.

Corresponding editor: M. NEI