POPULATION GENETICS OF ALLOZYME VARIATION IN *NEUROSPORA INTERMEDIA*[1]

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ABSTRACT

Electrophoretically detectable variation in the fungus *Neurospora intermedia* has been surveyed among isolates from natural populations in Malaya, Papua, Australia and Florida. The principal result is a pattern of genetic variation within and between populations that is qualitatively no different than the well documented patterns for Drosophila and humans. In particular, there is a high level of genetic variation, the majority of which occurs at the level of local populations. Evidence is presented which argues that *N. intermedia* has a population structure analogous to that of an annual vascular plant with a high level of vegetative reproduction. Sexual reproduction appears to be a regular feature in the biology of the species. Substantial heterokaryon function seems unlikely in natural populations of *N. intermedia.* Theoretical considerations concerning the mechanisms underlying the observed pattern of variation most likely should be consistent with haploid selection theory. The implications of this constraint upon the theory are discussed in detail, leading to the presentation of a model based upon the concept of environmental heterogeneity. The essence of the model, which is equally applicable to haploid and diploid situations, is a shifting distribution of multiple adaptive niches among local populations such that a given population has a small net selective pressure in favor of one allele or another, depending upon its particular distribution of niches. Gene flow among neighboring populations with differing net selective pressures is postulated as the principal factor underlying intrapopulational allozyme variation.

THE fungus *Neurospora crassa* has enjoyed a long and illustrious career as the object of genetic investigations. It is a member of a small, elite group of organisms whose genetics are exceedingly well characterized. Yet population genetic studies of this species or its relatives are practically nonexistent. Valid reasons underlie this conspicuous omission, perhaps the largest being the lack of suitable means for assaying genetic variability in natural populations. Analyses of recessive lethals and chromosomal rearrangements, so long the backbone of population genetic studies with Drosophila, are not practical or possible with Neurospora. However, the advent of electrophoretic techniques for assaying

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allozyme variation in natural populations has opened the way for population genetic studies of Neurospora, just as it has done for many other species. Inclusion of the fungi as objects for the study of natural variation holds great promise for population genetics. The peculiarities of fungal biology—frequently involving substantial haploid states, heterokaryosis, and parasexual recombination as well as sexual recombination—are more varied and qualitatively far removed from the predominantly diploid life cycles of vascular plants and most animal species. Moreover, significant contrasts in life cycles can be found within congeneric fungal groups. Consequently, the study of fungal population genetics will provide increased generality to the accumulating body of population genetic data and promises to add a new dimension to the field of population genetics.

Over the past decade allozyme variation has become well characterized for a large number of organisms, most notably many species of Drosophila, vascular plants and numerous vertebrates including man. The list of workers is too numerous to quote. See, for instance, LEWONTIN (1973). Variability has been characterized within populations, between conspecific populations and between congeneric populations of varying degrees of taxonomic affinity. The accumulating data have had a profound influence upon theoretical population genetics. Although differences associated with the biochemical function of the gene (GILLESPIE and KOJIMA 1968; JOHNSON 1974) and with the ecology of the species (SELANDER and KAUFMAN 1973) have been found, the patterns of variation have been remarkably similar. However, practically all studies have used organisms which exhibit a predominantly diploid life cycle, which automatically makes for substantial theoretical similarity. Comparable studies with haploid species are almost totally lacking, even though electrophoresis has been used with fungi for many years by mycologists interested in taxonomic and physiological questions. See, for example, HALL (1973) and accompanying papers and GARBER and RIPPON (1968) for a general overview. The most relevant Neurospora studies are the taxonomic work of REDDY and THRELKELD (1971, 1972) and REDDY (1973). Their work is a notable start in the characterization of Neurospora allozyme variation. However, it, like almost all other allozyme work with haploids, is based upon relatively few natural strains and is not suitable for population genetic interpretation. The little evidence that is available is somewhat contradictory with respect to the pattern of intraspecific variability. The most reliable data are from MILKMAN's (1973) work with *Escherichia coli*, which strongly suggest that the pattern for that species is qualitatively no different than those for diploid species. On the other hand, the unpublished study of MCCLEMENTS and GARBER (quoted in GARBER and RIPPON 1968) found essentially no variation in a relatively large number of strains of *Aspergillus nidulans*.

This paper reports the results of a survey of allozyme variation in an extensive collection of natural isolates in *Neurospora intermedia*. This species, described by TAI (1935), hybridizes rather readily with both *N. crassa* and *N. sitophila*. It was first isolated from a corncob in Nanking, China in 1927. It appears to be the prevalent Neurospora species in many parts of the Far East (PERKINS and TURNER 1973; PERKINS, TURNER and BARRY 1975). It is also found in Hawaii

and the Gulf of Mexico region in the U.S.A. Blooms of *N. intermedia* are commonly found on vegetative stubble in recently burned grass and sugarcane fields (PERKINS 1970; PERKINS, TURNER and BARRY 1975). As the blooms age and the available nutrients are depleted, they enter the sexual phase and produce ascospores which, presumably, remain dormant until the next burn. The blooms produce abundant conidia which are subject to wide wind dispersal. The species can be found on such opportunistic substrates as old corncobs and temple offerings (PERKINS' collection list). An additional facet of *N. intermedia* is its apparent association with human culture in West Indonesia, where it, presumably, is the fungus kept in culture for use in the production of edible ontjom (HO, KOH and CHONG 1973). Since human culturing could have an effect upon the genetic variation present in cultured strains, none of the strains used in this study was obtained from ontjom. Likewise, the effects of utilizing opportunistic substrates is not of immediate importance to the strains in this study since all were collected from colonies which are presumed to have arisen from germinating ascospores.

Data are presented here for populations in Malaya, Papua, Australia and Florida. The principal result is a pattern of variation within and between populations that is indicative of a cohesive gene pool for the entire species and is indistinguishable from the usual patterns observed for diploid species.

MATERIALS AND METHODS

All strains used were collected by DAVID D. PERKINS between 1968 and 1970. The 18 sites are listed in Table 1. The number of isolates, PERKINS' stock numbers, and the substrates on which the strains were found are given for each site. Except for Papua all the sites are small areas such as a single field. The Papua isolates were collected from seven points along about twenty miles of Rouna Road, beginning at Rouna Falls (elevation just under 2,000') and extending down to the coast near Port Moresby. Except for the isolates at Rouna Falls only one or two isolates per point were obtained. For comparative purposes the five strains from Rouna Falls are listed as one site while the others are lumped together. The Kuala Lumpur site consisted of a burnt tree on the campus of the University of Malaya. The Kuala-Selangor Road and Klang Road isolates were obtained from six sequential sites on a circular route immediately northwest of Kuala Lumpur. The three Cairns and three Townsville sites lie within approximately five-mile spans near or within their respective cities. The Florida isolates are from two sites about thirty miles apart in central southern Florida.

Collecting had been accomplished by sampling conidia from well separated small colonies growing on burned substrate. A sterile toothpick was used to transfer the conidia to a sterile collecting envelope (PERKINS 1970). My strains were received either suspended on silica crystals (PERKINS 1962) or on slants freshly started from silica crystals. Most strains were obtained directly from PERKINS' lab; however, some came from the Fungal Genetics Stock Center. Strains were maintained on agar medium (VOGEL 1956) supplemented with 5% yeast extract (Difco) and 5% casamino acid (Difco) prior to being grown for electrophoresis. Growth was done as soon as possible to minimize the duration of laboratory culturing. Crosses were made on agar slants using the synthetic medium of WESTERGAARD and MITCHELL (1947). Strains were grown for electrophoresis in liquid culture of standard minimal medium (VOGEL 1956). Initially 750 ml media in a 2,500 ml wide-form shaker flask was used. It was later found that 150 ml medium in a 500 ml erlenmeyer flask produced sufficient yield. Growth was from three to six days on a platform shaker at room temperature. Spot tests revealed no differences in the electrophoretic results over this span of growth time. Grown cultures were harvested on cheese cloth and lyophilized overnight. Lyophilized material was kept refrigerated in a small screw-top bottles stored inside plastic containers.

TABLE 1

Sources of Neurospora intermedia *isolates analyzed*

Site		Number strains	Perkins' numbers*	Substrate
MALAYA				
Kuala-Selangor Road-1	(KSR-1)	6	P127–P233	Burnt grass
Kuala-Selangor Road-2	(KSR-2)	7	P134–P240	Burnt grass and brush
Kuala-Selangor Road-3	(KSR-3)	8	P241–P248	Burnt grass
Kuala-Selangor Road-4	(KSR-4)	7	P249–P255	Burnt grass
Klang Road-5	(Klang-5)	4	P256–P259 P261–P262	Burnt sedge
Klang-6		6	P263–P267 P269	Burnt grass
Kuala Lumpur	(KL)	7	P270–P276	Burnt tree
Singapore	(Sing.)	8	P277–P284	Burnt vegetation, incl. banana leaves
PAPUA				
Rouna Falls	(Rouna F.)	5	P32–P36	Burnt grass and brush
Rouna Road	(Rouna R.)	10	P37–P47	Burnt grass and brush
AUSTRALIA				
Cairns-1		10	P89–P98	Burnt grass and brush
Cairns-2		9	P99–P107	Burnt grass and lumber
Cairns-3		2	P109, P111	Burnt litter
Townsville-1	(Towns-1)	8	P113–P114 P116–P121	Burnt grass
Townsville-2	(Towns-2)	8	P122–P129	Burnt grass
Townsville-3	(Towns-3)	10	P131–P140	Burnt grass
Brisbane	(Brisb.)	11	P78–P88	Burnt grass on railway embankment
FLORIDA				
LaBelle		8	P405–P412	Burnt vegetation
Clewiston	(Clew.)	11	P413–P423	Burnt vegetation

* Some of the strains also have FGSC numbers. They are listed by site in the FGSC stock lists.

Such material has been successfully maintained for several years, provided it is kept free of moisture.

Samples were prepared by grinding .1 gm of lyophilized material with dry ice and a cold mortar and pestle, followed by suspension in 1 ml of 5% sucrose solution and centrifugation at approximately 15,000 g for 30 min. For the enzyme systems studied, such samples have been kept refrigerated or frozen up to three days without noticeable loss of activity. Gel pockets were layered with 15 μl of supernatant using a micro-syringe.

Electrophoresis was performed using vertical slab acrylamide gels made with 7% (w/v) cyanogum in gel buffer. All buffers were diluted to appropriate molarity from a stock solution of 1.0 M Tris-borate, pH 8.9. All gels were fixed in a solution of 5:5:1 water, methyl alcohol, glacial acetic acid. Details for the individual systems are as follows: *General proteins*: Both gel and electrode buffers were .1 M Tris-borate. Runs were at 200 v for 30 min followed by 400 v for 2 hrs. Staining was done with .03% (w/v) coomassie blue in 5:5:1 glacial acetic acid overnight at room temperature. Gels were destained with frequent changes of 5:5:1 glacial acetic acid. *Acid phosphatases:* Both gel and electrode buffers were .15 M Tris-borate. Runs were at 200 v for 30 min followed by 350 v for 2 hrs. Gels were incubated for 20 min in 100 ml

.1 M acetate buffer, pH 4.5 before adding the staining solution which consisted of 100 mg fast blue BB, .5 gm polyvinyl pyrallidane (K30), .5 ml of .1 M $MgCl_2$, .5 ml of 10% (w/v) $MnCl_2$, 10 ml of 20% (w/v) NaCl and 100 mg Na-α-naphthyl acid phosphate in 100 ml acetate buffer. *Esterases:* Gel buffer was .15 M Tris-borate with the addition of 5 ml of .1 $MgCl_2$ per 160 ml buffer. Electrode buffer was .15 M Tris-borate. Runs were at 200 v for 30 min followed by 300 v for 1.5–2 hrs and 400 v for 30 min. Staining was done with 40 mg α-naphthyl acetate, dissolved in 2 ml of 50% acetone, and 100 mg fast red TRN added to 200 ml of phosphate buffer, pH 6.5.

RESULTS

Photographs of representative gels assayed for general proteins, acid phosphatases and esterases are shown in Figures 1, 2 and 3, respectively. The pattern for general proteins is the most complex; bands corresponding to eight presumptive loci are indicated in Figure 1. The designations *GP-5* and *GP-6* were originally assigned to bands located between *GP-4* and *GP-7*. Unfortunately, staining in this area is so often smeared as to make scoring unreliable. *GP-1*, *GP-2*, *GP-7* and *GP-8* are monomorphic for *N. intermedia* with none or only one or two variants having been observed. *GP-3* is quite variable throughout the species; however, the variants have failed to behave in a Mendelian fashion and these bands are not used for the data of this paper. *GP-4* has alleles *100* and *95* exempli-

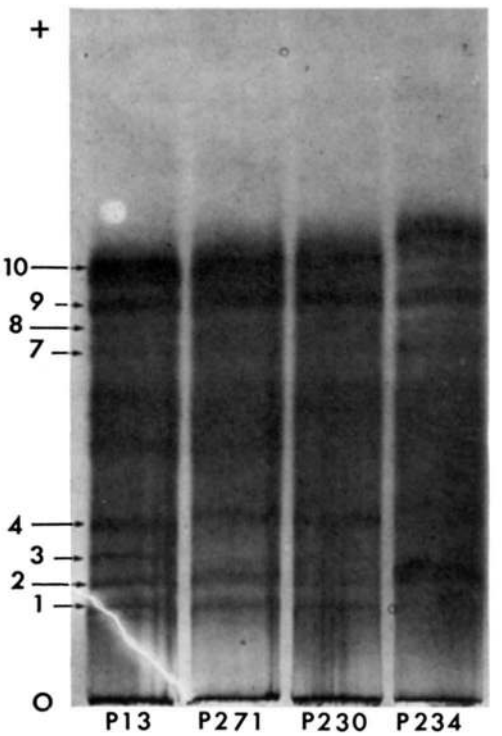

FIGURE 1.—Representative general protein banding patterns. Band designations are given at the side and strain designations are given at the bottom. The origin is marked with "O" and migration is anodal.

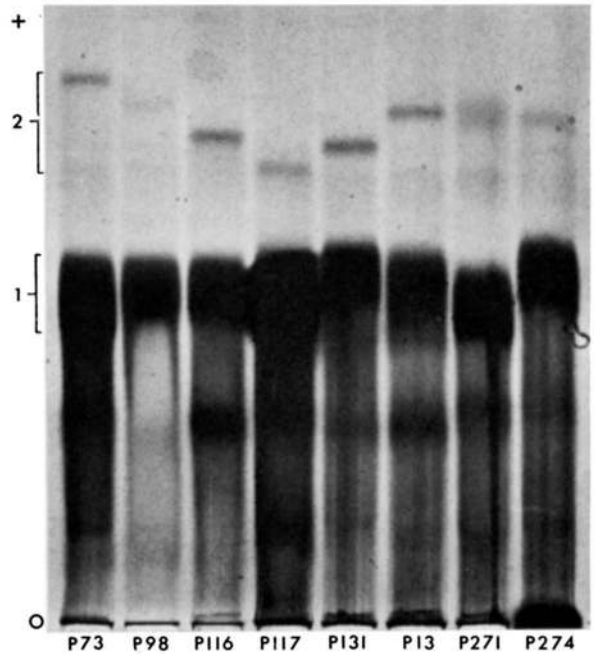

FIGURE 2.—Representative acid phosphatase banding patterns. The range for the two loci is given at the side. See text for particular allele designations. Strain designations are given at the bottom. The origin is marked with "O" and migration is anodal.

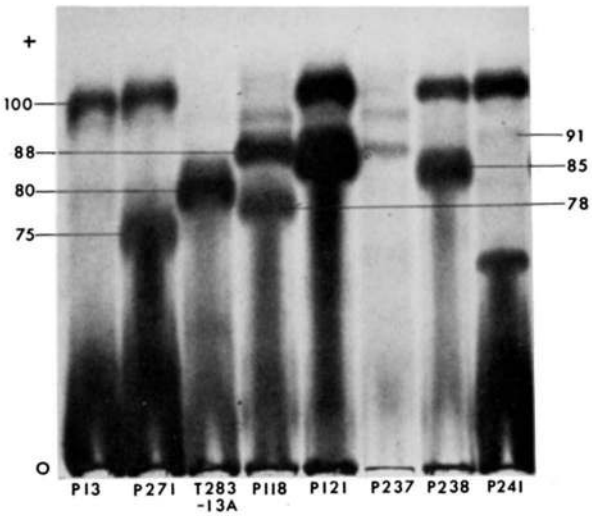

FIGURE 3.—Representative esterase banding patterns. The band designations are given at the sides. Strain designations are given at the bottom. T283-13A is not *N. intermedia*. The origin is marked with "O" and migration is anodal.

fied in Figure 1 by *P234* and *P230*, respectively. (Alleles are designated by their mobility relative to the most common form which is assigned the value *100*.) *GP-10* has three alleles: *100*, *106* and *L*. The first two are exemplified by P230 and P234, respectively. The last, which may be a null allele, is not shown. It has only light bands which are presumably the same as the light bands that can be seen in Figure 1 for P234 at the level of the *100* allele.

GP-9 is the locus with the greatest potential taxonomic value. The band shown in Figure 1 represents a monomorphism unique to *N. intermedia*. Non-intermedia strains typically exhibit a slow allele *94* that is at the same level as *GP-8*. Among the confirmed *N. intermedia* strains examined, a fast allele *106* was found for P30 (FGSC #1782) from Manila. Also, one presumptive null was found and the atypical allele *94* was found in two cases. Based upon the limited available interspecific data, the presence of the allele *GP-9(100)* can be taken as presumptive evidence of the strain's being *N. intermedia*.

The acid phosphatase loci are highly variable genetically. Two loci are assayable under the growth conditions used. These are indicated in Figure 2. Three alleles have been found for *AP-1*: *96*, *100* and *104*. These alleles are represented in Figure 2 by strains P271 for *96*, P13 for *100*, and P274 for *104*. The locus *AP-2* has a large number of alleles. The principal ones are *90*, *95*, *98*, *100* and *104*. Two strains in Florida, including PERKINS' reference strain P420 (FGSC #2316), have null alleles at this locus. Representative strains in Figure 2 are P117 for *90*, P131 for *95*, P274 for *98*, P13 for *100* and P73 for *104*. All these alleles show the usual Mendelian segregation pattern.

The esterases are highly variable and genetically complicated. In fact, their formal genetics still remains to be fully elucidated. REDDY and THRELKELD (1972) present evidence that two loci are involved in the difference between the principal band in *N. crassa* and the principal band in *N. sitophila*. The data for *N. intermedia* are compatible with their findings but more complex. Since the underlying genetics is not known, the data for the esterases are recorded as the presence or absence of bands, which are designated by their mobility relative to the most common band, prefixed by the letter "B" to emphasize that they cannot be interpreted as alleles. These data are included, in spite of their inadequate genetics, for two reasons. Firstly, the bands for a given strain are consistent even when there have been multiple cultures of the same strains, each started from a single conidium. Consequently, the data provide a valid indication of phenotypic variation within and between populations. Secondly, the data are comparable with the published results of REDDY (1973). Representatives of the various banding patterns are shown in Figure 3. The band B100 is the principal band for *N. intermedia* and corresponds to REDDY's (1973) band number 4. Band B80 corresponds to his band number 6, which is characteristic of *N. sitophila*. The heavy staining area near the origin can sometimes be resolved into three bands for which no demonstrable variants have been found. However, the heavy staining makes these bands unreliable for scoring and they have been ignored.

The primary purpose of this paper is the presentation of data concerning the intraspecific variation within and between populations of *N. intermedia*. Tables

2, 3 and 4 give the basic data for the variable loci. Table 2 additionally gives the distribution of the two alleles, *A* and *a*, at the mating type locus. (These latter data were kindly provided by DAVID PERKINS and BARBARA TURNER.) The numbers in these tables are the number of strains in each site that exhibit the given allele (or band in the case of esterases). Table 5 summarizes the data by grouping them together by major regions. The grouped data are expressed as frequencies given in percentages. For the mating type locus, mixed $A + a$ isolates are counted as contributing one of each allele. For esterases a problem arises since bands rather than alleles are scored. Consequently, one strain may have more than one band. The numbers in Table 5 give the percent of strains exhibiting the given band. Values for the species as a whole are calculated in two ways. The numbers in column "A" are simply the percentages for the total collection. Giving the figures in this way weights each site and each region proportionally to the number of strains collected in it, which may reflect more upon the collector's itinerary than upon the relative contribution of the area to the species as a whole. The numbers in column "B" are the unweighted averages of the values for each of the four regions. Both methods of calculation are somewhat arbitrary. However, the two columns are not in great disagreement and either can probably be taken as a first approximation of the true situation.

TABLE 2

Distribution of alleles at general protein and mating type loci

	Locus and allele							
	Mating type			*GP-4*		*GP-10*		
Site	*A*	*a*	*A+a*	*95*	*100*	*100*	*106*	*L*
KSR-1	2	3	1	4	2	6	0	0
KSR-2	4	2	0	1	5	5	1	0
KSR-3	3	5	0	2	4	5	1	0
KSR-4	4	3	0	3	4	7	0	0
Klang-5	2	2	0	1	3	4	0	0
Klang-6	2	4	0	1	5	6	0	0
KL	5	2	0	1	6	6	1	0
Sing.	2	5	1	1	7	6	0	2
Rouna F	2	1	2	0	5	4	0	1
Rouna R	6	4	0	1	8	8	1	0
Cairns-1	4	6	0	5	4	7	1	1
Cairns-2	4	4	2	1	8	0	0	9
Cairns-3	0	3	0	1	1	0	0	2
Towns-1	6	3	1	6	2	3	0	5
Towns-2	4	5	0	7	1	3	0	5
Towns-3	6	4	0	2	8	6	0	4
Brisbane	4	6	0	10	1	7	1	3
LaBelle	5	3	0	4	2	8	0	0
Clewiston	6	5	0	7	4	11	0	0

TABLE 3

Distribution of alleles at acid phosphatase loci

Site	Locus and allele								
	AP-1			*AP-2*					
	96	*100*	*104*	*90*	*95*	*98*	*100*	*104*	Other
KSR-1	0	3	3	1	0	0	3	1	1
KSR-2	0	3	3	2	0	1	3	0	0
KSR-3	0	4	3	1	1	0	5	0	0
KSR-4	0	5	2	1	0	1	5	0	0
Klang-5	1	1	2	0	0	0	3	1	0
Klang-6	0	4	2	0	0	0	4	2	0
KL	1	5	1	1	0	1	4	1	0
Sing.	1	6	1	0	0	1	7	0	0
Rouna F	0	4	1	0	1	0	3	1	0
Rouna R	0	7	2	2	3	0	2	2	0
Cairns-1	1	4	4	1	5	0	3	0	0
Cairns-2	0	1	8	0	0	0	9	0	0
Cairns-3	0	1	1	0	0	0	2	0	0
Towns-1	0	6	2	2	5	0	1	0	0
Towns-2	2	4	2	2	5	0	1	0	0
Towns-3	0	5	5	1	5	0	2	1	1
Brisbane	1	5	5	1	5	1	4	0	0
LaBelle	1	4	3	0	0	0	7	0	1
Clewiston	1	8	2	0	0	0	10	0	1

TABLE 4

Distribution of esterase bands

Site	Band						No. strains scored
	B100	B91	B88	B85	B80	Others	
KSR-1	4	0	0	2	0	0	6
KSR-2	6	0	0	2	2	0	6
KSR-3	4	1	0	2	0	1	6
KSR-4	7	0	0	3	0	1	7
Klang-5	2	2	0	1	0	0	4
Klang-6	5	0	0	2	0	0	5
KL	5	0	0	3	1	1	7
Sing.	3	3	0	4	2	2	8
Rouna F	5	0	1	2	0	1	5
Rouna R	9	1	1	2	0	1	9
Cairns-1	5	2	4	1	0	4	7
Cairns-2	9	0	0	0	0	1	9
Cairns-3	2	0	0	0	0	0	2
Towns-1	5	1	3	0	0	3	8
Towns-2	6	2	2	0	0	0	6
Towns-3	9	0	1	1	2	5	12
Brisbane	9	2	1	1	0	0	9
LaBelle	7	0	0	1	0	0	8
Clewiston	11	0	0	0	0	0	11

TABLE 5

Frequencies in percent grouped by major regions

Locus	Allele	Region				Species total	
		Malaya	Papua	Australia	Florida	A	B
M.T.	A	48	59	48	58	50	53
	a	52	41	52	42	50	47
GP-4	95	28	7	56	65	42	39
	100	72	93	44	35	58	61
GP-10	100	90	86	46	100	73	81
	106	6	7	4	0	4	4
	L	4	7	51	0	23	16
AP-1	96	6	0	7	11	6	6
	100	61	79	46	63	57	62
	104	33	21	47	26	37	32
AP-2	90	12	14	12	0	11	10
	95	2	29	44	0	21	19
	98	8	0	2	0	4	3
	100	67	36	39	89	55	58
	104	10	21	2	0	6	6
	Other	2	0	2	11	3	4
Est	B100	73	100	85	95	84	88
	B 91	12	7	13	0	10	8
	B 88	0	14	21	0	10	9
	B 85	39	14	6	5	20	16
	B 80	10	0	4	0	5	4
	Other	10	14	25	0	15	12

DISCUSSION

Data

It is immediately apparent from the above results that the gene pool of *N. intermedia* contains substantial genetic variability and that the variability exists, for the most part, within local populations. In other words, the pattern for this fungus is the same as those for man, mice and flies.

Before discussing the pattern in detail, however, some matters of fungal biology need consideration. One of the principal and immediate problems confronting the fungal population biologist is deciding what constitutes individuals and populations (see, for example, BURNETT 1968, chapter 17). For the reasons that follow, the answers to this problem for *N. intermedia* are probably no different than for, say, an annual vascular plant capable of extensive vegetative reproduction.

Consider the matter of individual identity, particularly with reference to the individual strains in the collection. A single ascospore together with the vegetative growth stemming directly from it certainly fits the standard concept of an individual. Ideally one would like each natural isolate to have an immediate vegetative history that traces back to a single ascospore unique to that isolate.

For the collector confronted with existing vegetative blooms it is impossible to know with certainty whether the individual blooms conform to this ideal. Departures from the ideal can occur in two directions. On the one hand, different blooms may be sister clones, stemming from the same ascospore *via* conidial propagation. Whether they should be considered different individuals is debatable; but, in any event, it is desirable to know if one is dealing with such a situation. On the other hand, a single bloom may be a mixture of two or more different individuals in the ideal sense. Moreover, the mixture may be one of pure physical intertwining of mycelia or it may be one involving hyphal fusions and heterokaryosis. In retrospect it appears that none of these complications greatly affects the collection used in this study. The genetic distinctness of different strains is overwhelmingly demonstrated by the fact that, almost without exception, any two strains from the same site exhibit a difference at at least one of the loci studied. The question of mixed mycelia could have been completely excluded by using single vegetative spores (conidia) to start each strain. This was not done; however, the data on mating types indicate that, while some mixing is present in the collection, it is at a relatively low level. In particular, Table 2 shows that about 5% of the isolates contained both mating types. So, perhaps 10% of the strains came from mixed conidia. However, the effect of mixed strains is probably much less. "Heterozygote" banding patterns, which would be indicative of genetically mixed isolates, were notably rare.

The matter of heterokaryon formation is more difficult and of greater significance. The absense of strains heterogeneous at individual loci is fair evidence heterokaryosis has probably not occurred among the strains within the collection. However, the collection method was designed to favor young blooms that might not have had opportunity for heterokaryon formation. The possibility that heterokaryosis becomes significant later in the bloom is a different matter. There is little doubt that the capacity for heterokaryon formation is widespread throughout the fungi (e.g., PARMETER, SNYDER and REICHLE 1963). The ability of *N. crassa* to form forced heterokaryons in laboratory cultures is well known. However, CATEN and JINKS (1966) have raised serious doubts as to the extent that the phenomenon occurs in natural populations, particularly in the ascomycetes. *N. crassa* is known to be polymorphic at a number of loci, including the mating type locus, each of which will prevent heterokaryosis between two strains that carry different alleles (e.g., WILSON and GARNJOBST 1966). MYLAK (1975) adapted the partial diploid technique of PERKINS (1968, 1975) for ascertaining vegetative incompatibility at four specific loci. His analysis of 15 isolates from three Louisiana populations of *N. crassa* revealed that all but two of the possible pairwise combinations contained at least one allelic difference at the loci studied, and are therefore incapable of heterokaryon formation. The loci studied are only four of many loci known to confer incompatibility. This result, if generally true for other heterothallic Neurospora species, has profound theoretical implications. It rules out any general possibility for genetic heterozygosity for nuclear genes in vegetative hyphae.

From the above discussion we can conclude that the various sites are local populations at least in the sense that they are a collection of genetically distinct individuals. However, the standard concept of a population involves an element of genetic interaction among individuals through the process of sexual reproduction. The mating type data suggest that populations of *N. intermedia* conform to the standard concept in this respect too. In all sites the two mating type alleles occur with remarkably even frequencies. The collection strategy alone is sufficient to explain this phenomenon: the recent burn at each site would have destroyed any vegetative colonies, while providing the stimulus to cause extant ascospores to break dormancy. A random collection of ascospores is necessarily expected to exhibit equal numbers of the two mating types. This argument, however, necessarily presupposes that sexual reproduction occurs with sufficient regularity to maintain a reasonable supply of ascospores in the soil. It is probable that the matter is biologically more profound than being merely the consequence of the collection strategy. A fungus adapted to living either parasitically upon live plants or, as is *N. intermedia*, saprophytically upon decaying vegetation must contend with an environment that is very patchy in both space and time with respect to suitable habitats for vegetative growth that are undoubtedly ephemeral. While spatial patchiness is probably irregular and unpredictable, temporal patchiness, such as occurs in a field that is burned once or twice a year, may be relatively regular. Consequently, dormancy is probably as important, if not more so, than dispersal in the ecology of such species. Dormancy is a necessity if a population is to maintain itself *in situ* for any length of time. The circumstantial evidence is convincing that ascospores provide the principal means of dormancy for Neurospora. They are known to be resistant to breakdown in soil and, compared to conidia, are more resistant to toxic cations and generally more durable (SUSMAN 1968). Heat shocking as a cue for initiating germination is an obvious adaptation to the observed ecological strategy of exploiting burned material. Finally, the even distribution of mating types in every site studied, including the otherwise nearly monomorphic Cairns-2, is evidence that populational continuity *via* dormant ascospores does, in fact, occur regularly. The point to be made, therefore, is that sexual reproduction appears to be an important factor in the biology of *N. intermedia*, not because of any intrinsic advantages associated with sexual reproduction (many other fungi have pretty well abandoned it) but rather because of its utilization as a means for dormancy. Of course, given the dependence of the species upon sexual reproduction, for whatever reason, population genetic consequences intrinsic to sexual reproduction will play a role in the biology of the species.

Genetic variation within local populations is commonly measured by the percent of polymorphic loci and the average heterozygosity per individual. The data here are too few both in number of individuals per site and in number of loci to allow meaningful calculations of these two summary statistics. Moreover, since genetic variability is correlated with gene function (JOHNSON 1974), these two statistics depend upon the particular loci studied, even when (as with these data) the choice of loci is purely pragmatic. Comparisons with other organisms are best

made for each particular enzyme system. Done this way, the agreement between Neurospora and, say, Drosophila is striking. The acid phosphatases and esterases, both of which are highly variable in these data, are systems that are likewise highly variable in Drosophila (JOHNSON 1974). General proteins, on the other hand, exhibit less and more subtle variation in both groups (e.g., LEWONTIN and HUBBY 1966). It can be reasonably concluded that the amount of genetic variability within local populations of *N. intermedia* is on the same order as that of Drosophila.

In addition to revealing substantial genetic variation within local populations of *N. intermedia*, the data provide a direct means for assessing variation between different sites, regions and countries. Qualitatively, the patterns are much the same throughout the species' range. The same array of alleles is found, for the most part, in all sites. However, the gene pool of the species as a whole cannot be considered uniform. There is one highly exceptional site and statistically significant quantitative differences exist in the distributions of some alleles. Notably, the allele *AP-2(95)* is common in Australia but rare or absent elsewhere. The esterase band Est(B88) is lacking in the Malaysian collection but has a moderate frequency in Australia and Papua. The Florida strains are notably uniform at the *AP-2* and esterase loci. The allele *AP-2(100)* is found in all Florida strains except the two, mentioned above, which have null alleles. The reduced variation at these loci suggest that the Florida sites may be less variable than the Far Eastern ones. It is worth noting that Florida is far removed—even for a readily dispersing species—from the major region of *N. intermedia*'s distribution.

On smaller geographical scales genetic homogeneity exists among the sites of some regions but not others. There is a striking degree of uniformity among the Malaysian sites. With one exception all the sites, including Singapore which is some 200 air miles south of the others, are statistically homogeneous for all loci. The exception is *Est(B100)* which is absent in five out of eight Singapore isolates while being present in 33 of the 40 other Malaysian strains. The Papua and Florida collections are small, but there is no indication of any spatial differentiation in either. In Australia statistical heterogeneity among the sites exists for almost all the polymorphic loci. Most of the heterogeneity is between sites of different cities, which is not surprising since the three cities span a range of close to 1,000 miles. Even within the Cairns and Townsville collections some differentiation occurs; for example, Townsville-3 differs significantly from the other two sites with respect to *GP-4*.

The most unusual site in the entire collection is Cairns-2. The nine strains from this site exhibit almost no variation for all loci except the mating type locus. Moreover, the alleles found at this site are not necessarily the ones that are most common in other Australian sites. The low level of variation at this site fits the pattern to be expected if there is local adaptation for different allozymes. The site is, in fact, unique; it is the edge of a lumber yard where scraps are being burned more or less continuously. The strains were collected from both burnt grass and burnt lumber. However, if the lack of variation is to be attributed to local adaptation, the obvious polymorphisms in all other sites require one to suppose that the

physical and biological conditions at Cairns-2 constitute an unusual environment for *N. intermedia* with respect to every one of the otherwise polymorphic allozyme loci. A more plausible explanation for this particular, atypical situation is that the strains at Cairns-2 stem from a recent colonization by a very limited number of propagules and that the population genetic situation at this site reflects a recent historical founder effect rather than an equilibrium distribution of allozymes. The equality of mating types implies that there has been at least one round of sexual reproduction within the site; that is, the isolates are not themselves the colonizers. It should be noted that, by this view, this site must represent a very rare event. Given the necessity of both mating types being present for a colony to become permanently established and the high level of polymorphism in local populations, we should generally expect new colonies to have considerable variation from the start. Moreover, dispersal of conidia should supply new colonies with additional variability within a very short time, thereby obliterating original founder effects.

Theory

The data presented above provide a clear, straightforward description of the pattern of genetic variation for *N. intermedia*. The challenge is to delve a step further and to speculate as to the population genetic factors responsible for effecting the observed pattern. The facts to be accounted for are two: (1) a high level of genetic variation within local populations, and (2) a lack of sharp dissimilarity between different populations.

The interpopulational pattern by itself can be attributed to a variety of plausible and theoretically sound explanations. The general species-wide genetic similarity undoubtedly is primarily attributable to the housekeeping role of natural selection. In terms of their direct effects upon Neurospora biology, the local environments of any two collection sites are probably more alike than different. The allelic state of a *randomly chosen* locus is more likely to reflect factors associated with being a Neurospora in general than with being a Neurospora in a particular local environment that differs in some particular ways from other local Neurospora environments. In short, genetic similarity among related individuals (even of different species) is to be expected. Of course, local environments do differ. Australia is not Malaya. Local adaptation is the very backbone of micro-evolutionary theory. Consequently, the observed pattern of small, but significant, genetic differences between populations, with differences generally increasing with increased geographic separation, is fully compatible with population genetic theory.

The difficulty arises when one attempts to incorporate the observed high level of local variation into the above model. The theory is straightforward: natural selection promotes the locally more adapted genotype. For diploid organisms and heterokaryotic organisms the favored genotype may be one that is heterogenic. For haploid organisms the basic selection theory unequivocally predicts fixation. With heterokaryosis ruled out for *N. intermedia*, selection during vegetative growth must fall into the latter category. However, the possibility of significant

diploid selection cannot be excluded. The importance and regularity of sexuality in the overall biology of *N. intermedia* have been discussed in detail above. Perithecia involve complex morphogenesis, are of substantial duration and are, undoubtedly, fully subject to selection, including potential overdominant situations. Consequently, the applicable selection theory for this species is the sort considered by SCUDO (1967) that takes into account the effect of selection on alternating haplo and diplophases.

The essence of the theoretical considerations are as follow: Suppose the relative haplophase fitnesses are $1-r$ and 1 for A and a, respectively, while the diplophase fitnesses are $1-s$, 1 and $1-t$ for AA, Aa and aa, respectively. Let p denote the frequency of A at the time of fertilization. Assuming random mating, there is a mathematical equilibrium at the point $\hat{p} = (t-r)/(t+s-rs)$. If the selection coefficients are such that this point is either less than zero or greater than unity there is loss or fixation of A, respectively. Otherwise, either a stable or unstable polymorphic equilibrium is possible. There are two principal classes of potential polymorphic equilibria. First is the case in which one allele is favored in haplophase and the other in diplophase (r and t positive and s negative, or *vice versa*). In such case one might expect evolution to lead *via* gene duplication to two loci fixed for alternate alleles with one functioning during diplophase and the other during haplophase—in short, isozymes. If such has been the case for any commonly surveyed allozymes, appropriate studies with vegetative and perithecial materials should reveal them. Electrophoretic studies by NASRALLAH and SRB (1973) with perithecial material from several species of Neurospora revealed a novel general protein band. However, their data indicate that it is more probable that they have found a gene product unique to perithecia rather than an isozyme of a gene which is expressed in vegetative material. If the isozymes are to be found, studies with particular enzyme systems should prove more illuminating.

The second case is the situation in which overdominance exists in the diplophase (s and t both positive). Polymorphism is possible provided the effect of directional selection in the haplophase does not overpower the overdominance of the diplophase. The latter will prevail if the following inequality holds: $-s/(1-s) < r < t$. Roughly speaking, this inequality says that the magnitude of selection in the diplophase must be greater than the magnitude in the haplophase. For species such as Neurospora, with substantial gene action in both phases, there is no *a priori* reason to expect this either to be or not to be the case. Undoubtedly it will vary from locus to locus, depending in each case upon the significance of allelic differences in each of the two phases. The only prediction that can be made is that, compared to species such as Drosophila for which the effects of haplophase selection are negligible or absent, the effect of overdominance on the overall level of polymorphism ought to be diluted by virtue of haplophase selection prevailing at a fraction of the overdominant loci. Unfortunately, a far more extensive study would be required to establish whether the level of polymorphism in *N. intermedia* is, say, only half as large as in Drosophila.

Even though overdominant diplophase selection cannot be excluded for *N. intermedia*, there is justification for requiring that any general explanation of the pattern of variation conform to the requirements of haploid selection theory. In the first place, it seems reasonable that haplophase selection ought to dominate the behavior of some, if not many, allozyme loci. Secondly GILLESPIE and LANGLEY (1974) have argued that allozyme loci in diploid species are most likely governed by genic selection, which is, for the most part, equivalent to haploid selection. Finally, the overall similarity of the patterns of variation for Neurospora, Drosophila and, it would seem, *Escherichia coli* (MILKMAN 1973), suggests that the general explanations for these three dissimilar taxa ought to be, for the most part, alike. With respect to selection, haploid theory may be taken as the common dominator. In so doing, the error is in the direction of underestimating the ability of a species to maintain local polymorphism. In particular, overdominance is excluded as a major cause of allozyme polymorphism, even though heterosis at the level of chromosomal arrangements is a firmly established empirical fact for Drosophila. Likewise, epistasis *per se* is an unlikely explanation for local polymorphisms. Epistatic interactions can, and most likely do, influence the dynamics of haploid selection; however, there is no known theoretical haploid situation for which they can produce a stable polymorphism. The well known diploid models such as those described by LEWONTIN (1974, chapter 6) all involve marginal overdominance at the equilibrium points.

Perhaps the most obvious and readily available explanation that is independent of differences between haploid and diploid selection schemes is provided by the extensive theory for the stochastic behavior of selectivity equivalent alleles produced by continual mutational events. However, application of neutral allele theory to Neurospora encounters the same difficulty as with Drosophila, namely, joint consideration of observed patterns of intra- and interpopulational variation gives a poor fit with the theory. The equilibrium relationship of neutral genetic differentiation between populations to that within populations is well defined (see, for example, SPIETH 1974). For a readily dispersing species such as *N. intermedia* the theory clearly predicts (1) genetic variation between populations that is only slightly more than that within populations, (2) an effective population size that is on the order of the size of the entire species, and (3) an effective number of different alleles that is roughly the product of the effective population size times the mutation rate. The first point fits the facts; the second is probably true; the third is extremely unlikely. However, theoretical corrections such as those of KING (1974) for electrophoretically indistinguishable alleles and a limited number of possible neutral alleles per locus greatly reduce the difficulties raised by the third point. In fact, they are reduced to the extent that neutral theory, while not totally satisfying, presents a sufficiently plausible explanation for the *N. intermedia* data that rejecting it out of hand would be unjustified.

By the same token, using these data to reject more than the simplest selection hypothesis is equally unjustified. There are at least two well-known selection models that are applicable to haploid as well as diploid situations and are capable of producing local polymorphism. One is the situation in which selection

coefficients are intrinsically dependent upon gene frequency. Unfortunately, such a model is so unspecific in terms of its parameters that to invoke it as a general explanation without providing a mechanism is to beg the question.

The most plausible and realistic selection model appears to be one that is based upon gene flow over a heterogeneous environment in which one allele is favored in some areas and not in others. Note that the concept of environmental heterogeneity is used here on a conceptually different level than the way it was used earlier in reference to the presence or absence of suitable Neurospora habitats. Laboratory experiments with Drosophila by POWELL (1971) and by MCDONALD and AYALA (1974) produced increased allozyme heterozygosity with increased environmental heterogeneity. However, to uncritically invoke environmental heterogeneity is theoretically unsound. If it is to be a general explanation for the observed pattern of variation in Neurospora, theoretical considerations put definite restrictions upon the interactions of gene flow and selection.

Two forms of environmental heterogeneity are generally recognized: simple spatial variation and stochastic variation over space and time. The latter has been extensively investigated by GILLESPIE (1924; GILLESPIE and LANGLEY 1974, and references therein). His essential result is that polymorphism can occur if the environmental heterogeneity (i.e., the variance in fitnesses) is large compared to the average selection intensity. However, he showed that temporal variation alone is insufficient to maintain polymorphism in haploid populations. Rather, it is necessary that temporal variations be coupled with spatial heterogeneity such that temporal variations in one niche are not fully correlated with those in other niches. That is, simple spatial heterogeneity must be generally present. In what follows, attention will be restricted to the spatial case. The possibility of temporal variation will, to some extent, increase the likelihood of polymorphism.

When applying theory for spatial heterogeneity it is essential to take into account the patchiness of the environment relative to local populations. That is, do selection coefficients alternate signs with a frequency (over space) that is large or small compared to the geographical size of a local population? The concept of patchiness, therefore, depends upon the relationship between the environment and selection upon a given locus. A spatial patch is a geographical area within which selection is uniform with respect to allelic differences at the locus in question. The possibilities for patch size stretch across a continuum from one extreme of multiple adaptive niches randomly distributed within each local population to the other extreme of isolated populations occupying differing environments. The theoretical expectations vary with the spectrum.

The basic parameters in the theory of environmental heterogeneity are the strength of selection and the amount of gene flow between patches for which selection is in different directions. The latter is minimized in the case of an isolated population inhabiting a single patch or a population in the interior of a very large patch; the obvious expectation is monomorphism for the favored allele. For populations at the point of contact between two large patches the expectation is not so obvious; however, simple theory, such as given by LI (1955, chapter 21, section 5), predicts essential monomorphism if the magnitude of selection is

larger than the magnitude of migration. Sophisticated analyses and computer work such as those of SLATKIN (1973) and ENDLER (1973) predict that clines in gene frequency at the borders of large patches will be comparatively steep. Consequently, large scale environmental heterogeneity leads to a general expectation of monomorphism for the locally favored allele in each patch—that is, polymorphism in the species as a whole, but monomorphism within populations. This is clearly not the case for *N. intermedia.*

The gist of the theory is that the existence of polymorphism at the populational level depends upon patch size and selection coefficients meeting appropriate conditions. Local monomorphism will occur if patch size is too big or if it is too small and the selection coefficients fail to meet the appropriate conditions for multiple niche polymorphism. This clearly presents the greatest difficulty with invoking environmental heterogeneity as the mechanism underlying allozyme variability. Gene flow obviously must be the same for all loci while selection coefficients and patch size ought to vary among loci. With so many polymorphic loci it is expected, therefore, that there ought to be a few loci for which either selection or patch size is sufficiently large to produce a patchwork of locally adapted monomorphisms. The data of AYALA *et al.* (1974) show that such loci can, in fact, be found with sufficiently large surveys. Their observation that such loci are often the same in different, related species is significant in that selection and patch size should depend primarily upon biochemical properties of the locus that are probably similar for related species.

There is, however, another way in which environmental heterogeneity might effect local polymorphism. It is most readily described in the form of a verbal model. Suppose a species is more or less continuously distributed over a comparatively large area. Assume that the environmental patch size is very small, fitting the form of multiple adaptive niches. Next, assume that selection differences between the various alleles in each patch are small. The selection coefficients need not meet the requirements for multiple niche polymorphism. If they do, polymorphism will automatically occur. If they fail to meet the requirements the result will be a net selective pressure within the population as a whole tending to favor one allele or another. For the population, therefore, each allele will have an effective selection coefficient that is an appropriate average of the values in the different niches and which will be smaller in magnitude than the coefficients in the individual niches. The final and crucial element in the model is the supposition that the distribution of niches differs from one population to the next, so that in some populations the net effect is a weak favoring of one allele while in others it is the favoring of another allele. These weak, but opposite, net selective pressures in neighboring populations together with gene flow among the populations are seen as the principal factors in the maintenance of local polymorphisms.

What is proposed, therefore, is a model of shifting multiple niches that creates a variance in net selection coefficients among interacting populations. Differences among neighboring populations are more likely to reflect quantitative differences in the distribution of the niches rather than qualitative differences in the kinds of

niches. This may even hold true over relatively large geographical areas such as the entire Kuala Lumpur region. Over larger areas one would expect that qualitative differences might arise. For example, the absence of *AP-3(95)* in Malaya may reflect an absence of niches favorable to it in Malaya but present in Australia. The model has been presented in the form of spatially varying distributions of the niches. Temporal variations of the distribution of niches within individual populations are reasonable to expect and should further contribute to the maintenance of genetic variability.

The assumption that the selection coefficients are small is highly plausible for allozymes. It is well known that the vast majority of mutants other than lethals have small effects on fitness. Moreover, what we are dealing with is a matter of biochemical fine tuning, not gross adaptive shifts of the sort that morphological differences can confer. With the exception of null alleles, which have a negligible role in allozyme data, and possibly some general protein loci, all electrophoretically detectable alleles are functional molecules. Presumably the species could exist quite happily with any one of them alone. Consequently, the most likely relevant form of selection is a truncation model wherein the allozyme loci act as polygenes upon the organism's fitness in a given niche. It is known from quantitative genetic theory (e.g., FALCONER 1960, chapters 11 and 12) that for a given selection intensity the selection coefficient for each locus is roughly inversely proportional to the square root of the number of loci and should, therefore, be relatively small. Epistasis could further reduce the effective strength of the selection coefficients. If a particular gene is favored in conjunction with certain alleles at other loci and not favored with others, the overall effect on the locus will be some sort of average between the extremes.

An interesting aspect of the model is that its emphasis upon weak selection brings the "selectionist" and "neutralist" positions into very close proximity. In terms of one's point of view concerning the biochemical and physiological consequences of small changes in protein structure, the difference between the two is one of degree. Perhaps this has something to do with the fact that both, with appropriate caveats, can be invoked to account for the observed data and neither can be unequivocally dismissed. There are, however, several reasons to prefer this model over the neutral theory. Firstly, the neutral theory pertains to polymorphic loci with selection coefficients that are smaller than the reciprocal of the effective population size. Given that effective population sizes often tend to be on the order of the entire species' numerical size, even nearly neutral alleles will be influenced primarily by selective differences. Secondly, this model has the property of accounting for minor variations in allozyme patterns in a way that is consistent with associated differences in the biology of the situation. For example, GILLESPIE and LANGLEY (1974) cite a number of situations in which observed heterozygosity correlates with biological predictions about the likelihood of multiple adaptive niches existing.

In conclusion, it should be emphasized that the model of shifting multiple niches is presented primarily as a plausible and realistic resolution to the theoretical difficulties posed by the growing body of allozyme data. It may, or may not,

prove a general explanation. Even if it does not, the phenomenon with which it grapples will still remain. Namely, the data from this study with *Neurospora intermedia* clearly show that high intrapopulational genetic variation at the molecular level is not an exclusive property of species with life cycles that constrain the effects of selection almost entirely to the diplophase.

It is interesting to speculate as to what extent the apparently regular occurrence of sexual reproduction influences the maintenance of high genetic variability in local gene pools of *N. intermedia*. To find the answer will require more comparative studies of variation—a task to which the fungi are eminently suited. In any event, the observation, made so forcibly for human data by LEWONTIN (1972), that the vast majority of the variability of a species is found within local populations has been extended one step further toward becoming a general property of biological systems.

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