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Enzyme Polymorphism in Plant Populations

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This review encompasses a decade of studies of enzyme polymorphism in plant populations, in the light of both general theory and specific, simplified models. The patterns of the observed frequency of heterozygotes, compared with panmictic expectations adjusted only for inbreeding, are summarised for 23 outbreeding and 7 inbreeding plant species. There is a trend for outbreeders to show less heterozygosity than expected, and inbreeders to show more, despite the contrary evolutionary pressures on the mating system (the so-called heterozygosity paradox). An annual life cycle and pollination by animal vectors seem to increase the discrepancy in outbreeders. Of the several forces which might account for this paradox, the effects of intense microgeographic differentiation, of low gene flow, of self compatibility and of overdominance of linked segments are predominant. The evidence indicates that inbreeding plant species show more intense geographic and microgeographic differentiation, and more intense multilocus associations than outbreeders. Recent attempts to describe selection operating on variants by the analysis of life cycle components, of physiological processes, and of genetic demography are discussed. The fundamental importance of mating systems and their variation, as a distinctive feature of plant populations is already clear from the studies in hand. Therefore a closer integration of the joint microevolution of mating systems, and of genetic variation is required in both theoretical and experimental studies.

INTRODUCTION

In 1974 Lewontin wrote "With their immense variety of breeding systems, plants will be extremely important for comparative studies, and for sorting out the forces influencing allozyme variation," and "In proportion to their potential, plants have been greatly neglected as materials for studies of genetic variation." This is not an isolated assessment. Simultaneously, a group of evolutionists were meeting at Missouri to review priorities in evolutionary research, and their report gave strong emphasis to the need for more work on plants (Anon, 1974). Indeed this report was highly critical of further allozyme surveys in animals which were not explicitly aimed at a specific evolutionary problem. From this criticism, however they excepted such survey work on plants in which variation surveys were much rarer. Thus the impression given

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by population geneticists was that there would be a favourable audience for work on plants which was long overdue.

However an alternative perspective is arguable. What has characterized allozyme studies in plant populations over the past decade has not been a preoccupation with the question of how much variation, but rather an emphasis on "problem orientation"—precisely that aspect which the Missouri workshop found lacking in much animal work. These problems have included—

(i) The effect of the mating system on genotypic structure and variation of populations (e.g., Marshall and Allard, 1970a, Solbrig, 1972).

(ii) The extent of multilocus correlations in allelic state in gametes and zygotes (e.g., Clegg *et al.*, 1972).

(iii) The degree of microgeographic differentiation within a population in the face of gene flow (e.g., Hamrick and Allard, 1972).

(iv) The patterns of geographic differentiation in relation to environment (e.g., Clegg and Allard, 1972).

(v) Analytical components of selection and genetic demography (e.g., Clegg and Allard, 1973; Schaal and Levin, 1976).

(vi) Relation between allozyme, and morphological variation (e.g., Marshall and Allard, 1970b).

(vii) The physiological effects of allozyme variation at specific loci (e.g., Marshall *et al.*, 1973).

(viii) The impact of selection in agricultural populations (e.g., Brown and Allard, 1971; Allard *et al.*, 1972a).

(ix) Species comparison in space (broad vs narrow niched relatives; Babbel and Selander, 1974), and in time (descendants vs evolutionary progenitors; Gottlieb, 1973a,b).

This review will cover progress made on several of these questions. It is clear that the literature was already appreciable on some of these topics by 1974 when the above assessments were formulated. Therefore as Clegg (1975) pointed out, much of this data was either ignored, or dismissed on incorrect or rather superficial grounds. For example the evidence from *Avena barbata* concerning questions (ii) and (iv) above has been criticized on the grounds that selfpollination in this species makes effective population size relatively small (Nei, 1975), that there was virtually no recombination (Lewontin, 1974), or that there could even be two "semispecies" (Clarke, 1973). These criticisms take little account of the published facts that selfing at most reduces variance effective number to half that expected under panmixia (Nei, 1975), and the populations of *A. barbata* are conspicuously large; that recombinants and heterozygotes were at detectable levels; and that the genetics of the polymorphisms is well documented (Marshall and Allard, 1969). There is clearly

a general need for an appreciation of the current overall status of allozyme studies in plant populations, and this review is an attempt in that direction.

More recently several reviews of plant population genetics have appeared, including those of Hamrick (1978) on levels of allozyme variation and longevity, of Gottlieb (1977a) on the effect of speciation, of Jain (1976) on the evolution of inbreeding in plants, and Bradshaw (1972) on the distinctive features of plant evolution. Here we consider the relationship between theory and empirical data on specific problems of variation structure. A number of recent theoretical reviews by Felsenstein (1976), Hedrick *et al.* (1976, 1978), and earlier on inbreeding species by Allard *et al.* (1968) and Karlin (1968), also form part of the background.

A. Genotypic Structure and the Breeding System

Plant species exhibit a great diversity of breeding systems (Darlington and Mather, 1949; Fryxell, 1957; Stern and Roche, 1974; and Jain, 1976), with several modes defined by the origin of uniting gametes: selfincompatibility (homomorphic or heteromorphic), selfcompatibility with predominant outcrossing, and predominant selfpollination. These potentialities are effected by population density, the distribution of the floral organs (whether hermaphroditic, monoecious, dioecious), developmental sequences within flowers (whether protandrous, protogynous or cleistogamous) and between flowers (e.g., permitting geitonogamy despite protandry in *Eucalyptus* species) pollinator activity, and pollen competition. In addition, there are several kinds of asexual or apomictic reproduction, either vegetative or agamospermous. Furthermore, populations or species may display varying mixtures of these modes, the proportions of which can be under complex genetic and environmental control (e.g., Horovitz and Harding, 1972).

When we consider the genotypic arrays at polymorphic loci in a plant population, the first interest of the different breeding systems lies in their effect, compared with random mating on

(a) the current genotypic arrays $\{G_i^t\}$ at each locus, in particular the altered level of heterozygosity.

(b) The transition between generations $\{G_i^t\} \rightarrow \{G_i^{t+1}\}$ in conjunction with other evolutionary forces (selection, drift, migration).

(c) The degree of correlation in genotypic state between different loci, in particular the probability of multiple heterozygosity.

A second problem is that of the evolution of different breeding systems themselves, particularly in the light of their diverse effects on genotypic structure.

Studies of predominantly inbreeding species have been especially productive in understanding the forces moulding genotypic structure in plant populations (Allard *et al.*, 1968). The power of such studies traces in part to the behaviour and the sampling properties of various statistics under inbreeding. Consider Wright's fixation index (F) which is a convenient measure (Jain and Workman 1967) of genotypic structure at a single locus,

$$F = 1 - \frac{\text{Observed heterozygosity}}{\text{Heterozygosity predicted under panmixia}}$$

In the diallelic case (allele frequencies p, q) the sampling variance in a random sample of N (Rasmussen, 1964) is,

$$var(F) = (1 - 2F)(1 - F)^2/N + F(1 - F)(2 - F)/2pqN \qquad (1)$$

The value of F at which sampling variance is a maximum for a given gene frequency is ($p \neq 0.5$),

$$F = [3 - 10pq - (3 - 12pq + 4p^2q^2)^{1/2}]/(3 - 12pq) \qquad (2)$$

From (2), the region of highest sampling variance for F is ($0 < F < 0.3$) when gene frequencies are in the most experimentally useful range ($0.2 < p < 0.5$). This is the region of F occupied by outbreeding populations, and thus lies behind the noted insensitivity of the chi-square test for departure from Hardy-Weinberg proportions in such populations (Lewontin and Cockerham, 1959).

On the other hand, predominant inbreeders in which $F > 0.9$, have sampling variances for F of about a quarter their equivalent in outbreeding populations with the same gene frequencies. Furthermore, inbreeding acts as a preserver of information on genotypic structure between generations. One round of complete random mating replaces any deviations at individual loci with panmictic proportions.

Table I gives several estimates of fixation indices ($\hat{F}$) for allozyme loci in many plant species, classified into inbreeders or outbreeders. The parameter t is the estimated rate of outcrossing as opposed to self fertilisation, where available ($0 \leqslant t \leqslant 1.0$), or simply whether the species is self compatible (S.C.) or self incompatible (S.I.). In the last case, t is assumed to be 1.0. The parameter ΔF is defined by formula (8) below, as the difference between the observed fixation index, and the expected value of the inbreeding coefficient. The outbreeders were further divided according to their longevity, (Hamrick 1978), and their principal pollen vectors, where "animal" includes insects, birds and mammals. The two exceptional cases of highly negative estimates are kept separate. In *Lycopodium lucidulum*, the predominant and presumably prolonged apomictic reproduction, confuses the definition of individuals

(Harper and White, 1974). The complete structural hybridity due to translocation heterozygosity in *Oenothera biennis* is presumably the reason for high negative F, despite predominant self pollination. These two cases follow the classical (Darlington, 1958) expectations of the effects of apomixis and structural heterozygosity on genic heterozygosity.

For the two major classes of data, the average and the weighted (by polymorphic loci $\times$ populations studied) average is obtained. These figures summarise two remarkable generalizations which seem to hold in many of the studies and which their authors have commented upon to varying extents. These are

1. In *outbreeding* species, there is a general *deficit* of heterozygotes when compared with panmictic expectations.

2. In *inbreeding* species, there are commonly an *excess* of heterozygotes when compared with expectations under Wright's neutral inbreeding law.

The short-lived or herbaceous outbreeders tend to show a larger deficit than the tree species. The deficit was markedly less in the wind pollinated Vs animal pollinated examples. However there was heterogeneity within categories indicating that the number of cases so far examined is only sufficient to suggest trends.

The two main results stand superficially, in contrast to classical expectations that heterozygosity is favoured in outbreeding species, but of no special consequence in inbreeding species. Furthermore, these empirical generalizations are paradoxical when the question of the evolution of mating systems is considered. Thus if heterozygotes are *in fact* favoured under inbreeding, how can the species maintain such a high level of inbreeding and not produce the favoured types? Conversely, why is a high level of outcrossing maintained despite the fact that heterozygotes apparently are not favoured types? Such a situation runs completely contrary to the usual models of evolution of selfing vs outcrossing (Maynard Smith, 1977a). As a convenient short-hand therefore, I will term the above two empirical observations—the *heterozygosity paradox*.

A variety of mechanisms have been put forward to explain the heterozygosity paradox. We consider them here as a checklist for future investigations, first those possibilities of positive bias in outbreeders, and second of sources of negative bias in inbreeders.

(1A) *Unknown Mendelian Genetics of Marker Loci*

This is of course not an explanation of the fact, rather a statement that the observation, as recorded may be incorrect. Unless there is some independent evidence of normal phenotypic segregation, this possibility must always remain (Jones, 1977). This problem can be avoided when the experimental organism is chosen. Preference should be given to species which are readily crossed,

TABLE 1

Estimates of Fixation Index in Plant Populations

Species		Pollination By	Polymorphic Loci	Population	$\hat{F}$	t	ΔF	Authors
A. Outbreeders (i)	Annuals							
Clarkia biloba		*A*	5	3	0.18	S.C.	—	Gottlieb (1974)
Clarkia lingulata		*A*	5	2	0.50	S.C.	—	Gottlieb (1974)
Clarkia rubicunda		*A*	6	4	0.36	S.C.	—	Gottlieb (1973a)
Lupinus subcarnosus		*A*	5	8	0.32	S.C.	—	Babbel and Selander (1974)
Lupinus texensis		*A*	5	10	0.14	S.C.	—	Babbel and Selander (1974)
Phlox drummondii	wild	*A*	7	73	0.36	S.I.	0.36	Levin (1977)
Phlox roemariana		*A*	4	15	0.42	S.I.	0.42	Levin (1978)
Stephanomeria exigua ssp. carotifera		*A*	8	11	0.08	S.I.	0.08	Gottlieb (1975)
S. exigna ssp. coronaria		*A*	7	1	0.15	S.I.	0.15	Gottlieb (1977b)
Lolium multiflorum	seedling	*W*	4	1	0.08	0.82	−0.02	Mitton *et al.* (1978)
	adult				0.01		−0.09	
Zea mays		*W*	8	2	0.01	0.99	0.0	Brown and Allard (1970)
(ii) Biennials								
Hymenopappus scabiosaeus		*A*	5	14	0.08	S.C.	—	Babbel and Selander (1974)
Hymenopappus artemisiaefolius		*A*	5	12	0.06	S.C.	—	Babbel and Selander (1974)

(iii) Herbaceous Perennials								
Liatris cylindracea		*A*	15	1	0.43	S.I.	0.43	Schaal (1975)
Silene maritima		*A*	2	1	0.10	S.C.	—	Baker *et al.* (1975)
Lolium perenne		*W*	3	9	0.04	S.I.	0.04	Hayward and McAdam (1977)
(iv) Woody Perennials								
Eucalyptus obliqua	seed	*A*	3	4	0.12	0.76	−0.02	Brown *et al.* (1975)
	adult				0.00			
Eucalyptus pauciflora	seed	*A*	7	3	0.14	0.68	−0.05	Phillips and Brown (1977)
	adult				0.01			
Ficus carica		*A*	2	4	0.28	S.I.	0.28	Valizadeh (1977)
Abies lasiocarpa		*W*	3	1	0.06			Grant and Mitton (1977)
Picea engelmannii		*W*	3	1	0.07			Grant and Mitton (1977)
Pinus ponderosa	> xeric			5	−0.37			
	< xeric			6	−0.03			
		W	1			0.96	−0.20	Mitton *et al.* (1977)
	Sth facing			4	−0.24			
	Nth facing			4	−0.08			
Pinus sylvestris		*W*	3	3	0.11	0.93	0.07	Rudin *et al.* (1974) and Stern and Roche (1974)

Table continued

TABLE 1 (*Continued*)

Species		Polymorphic Loci	Population	$\hat{F}$	t	ΔF	Authors
B. Inbreeders							
Avena barbata	CSA	4	1	0.75	0.01	−0.22	Marshall and Allard (1970)
	SRB	3	1	0.80	0.08	−0.06	
	Geyers	4	1	0.88	0.02	−0.08	Allard *et al.* (1972)
	CSA	5	1	0.90	0.02	−0.06	
	CSA	5	1	0.81	0.02	−0.16	Hamrick and Allard (1972)
Avena fatua		6	3	0.88	0.01	−0.10	Clegg (1972)
Bromus mollis	xeric	5	5	0.62	0.08	−0.23	Brown *et al.* (1974)
	mesic	5	5	0.84	0.11	0.04	
Hordeum spontaneum	xeric	18	8	0.976	0.004	−0.02	Brown *et al.* (1978)
	mesic	21	18	0.975	0.021	0.02	
Hordeum jubatum		5	3	0.96	0.013	−0.01	Babbel and Wain (1977)
Phlox cuspidata		5	43	0.68	0.22	0.04	Levin (1978)
Lycopersicon pimpinellifolium	central/sth. Peru	8	12	0.58	0.11	−0.26	Rick *et al.* (1977)
	Nth. Peru	8	8	0.28	0.30	−0.22	

C. Miscellaneous

Oenothera biennis	4	26	−0.41	<0.05	Levin (1975)
Lycopodium lucidulum	5	16	−0.66		Levin and Crepet (1974)

Summary

		No. of species	Mean†	Weighted mean
Outbreeders —	annuals	11	0.23	0.31
	biennials	2	0.07	0.07
	perennial herbs	3	0.19	0.18
	perennial trees	7	0.05	0.03
	Wind pollinated	7	0.02	0.01
	Animal pollinated	16	0.22	0.27
	MEAN	23	0.16	0.26
Inbreeders		7	−0.08	−0.03

† Mean F for outbreeders, ΔF for inbreeders.

or where individuals can be assayed as members of naturally occurring progeny arrays.

(1B) *Null Alleles*

This is again essentially another technical problem stating that some of the phenotypic homozygotes are in fact genetic heterozygotes. Null alleles have been found in both inbreeders (Rick and Fobes, 1975) and outbreeders (Gottlieb, 1975). The difficulty of this problem is that a rare null allele will remain undetected as a homozygous null, in all but the largest of samples.

Consider a panmictic population segregating for two phenotypically dominant alleles A_1 and A_2 and one recessive null A_n allele, with frequencies p, r and $n(p + r + n = 1)$. The allele A_2 is rarer than allele A_1 $(p > r)$. Suppose a random sample of N zygotes is assayed and the homozygous null phenotype is not recovered in the sample and only two alleles are assumed. Then the apparent gene frequency of A_1 from the sample is $p(1 - n)$ and the apparent fixation index is,

$$F_a = 1 - \frac{2pn/(1 - n^2)}{2pn/(1 - n)^2} = \frac{2n}{1 + n} \tag{3}$$

Thus rare null variants can bias fixation estimates by an amount of twice their frequency. Suppose in a particular sample $F_a = 0.30$. This result could obtain when $n = 0.177$, which is an appreciable population frequency. In order that samples have a better than even chance of detecting the null homozygotes in such a population, at least 22 zygotes should be sampled. Detection with 0.05 level of confidence would require 95 zygotes. It is clear that in the sampling strategies commonly employed, considerable bias may pertain to F estimates from undetected nulls. One possible check on the presence of such a bias is to assay the selfed progeny of the rarest phenotypic homozygotes because, if nulls are present, these are the most likely $[(p + 2n):(r + 2n)]$ plants of being heterozygous null.

Whilst the existence of undetected null alleles is obviously a statistically plausible explanation of the heterozygosity paradox, positive evidence so far suggests it may not play such a role. The most compelling argument against the widespread existence of undetected nulls in outcrossing plants is that heterozygous nulls are not a common feature of animal populations, particularly highly vagile insect species (see Burns and Johnson 1967, for an exception).

(1C) *Dominant Alleles at Modifier Loci*

This recent proposal envisages that allelic variation at a locus other than the structural genes, leads to alteration of electrophoretic mobility by post translational modification of the protein (Finnerty and Johnson, 1978). As yet there are no data which examine this in plants.

(1D) *Wahlund Effect From Selective Microdifferentiation*

If the single sample unwittingly includes individuals from two or more genetically heterogeneous subpopulations, then the fixation index will be biased up in amount equal to the mean standardized or Wahlund variance (Cavalli-Sforza and Bodmer, 1971). Heterogeneity of subpopulation gene frequencies could arise from heterogeneity of selection pressure or very small neighborhood sizes within which random divergence may have occurred (see 1F below). Substantial heterogeneity between microsites must be present if random mating occurs *within* microsites, to explain only moderate biases in F. (Manwell and Baker, 1970). Thus, if two equally frequent subclasses are present and are to produce a positive bias of $+\Delta F$, they must differ at a diallic locus in gene frequency by more than $(\Delta F)^{1/2}$.

In the case of the mixed mating system, with constant outcrossing rate t, assume that K subpopulations are large and isolated with gene frequencies $\{p_i: i = ,..., K\}$, and variance σ_p^2 and mean $\bar{p}$. The assumption of a constant outcrossing rate is unlikely to hold in nature. It is done here merely to illustrate the effect of gene frequency variance alone. Further assuming that genotypic proportions within each are in local neutral inbreeding equilibrium, the observable overall fixation index in a mixed sample would be,

$$\begin{aligned} F &= 1 - [2\bar{p}\bar{q} - 2(1 - F_e)\,\sigma_p^2 - 2\bar{p}\bar{q}F_e]/2\bar{p}\bar{q} \\ &= (1 - F_e)\,F_{ST} + F_e \end{aligned} \tag{4}$$

where $F_e = (1 - t)/(1 + t)$ and $F_{ST} = \sigma_p^2/2\bar{p}\bar{q}$ is Wright's measure of subpopulation differentiation.

Equation (4) is analogous to Wright's formula of subpopulation differentiation and shows that for equivalent differentiation (F_{ST}), there is less scope in inbreeders than in outbreeders for the Wahlund effect to cause alterations to F.

(1E) *Restricted Neighborhood Size*

This model proposes that mating takes place between random individuals within neighborhoods, but the individuals are on average more genetically related than two random members of the entire local population (Schaal, 1975). Mating individuals may be closely adjacent in space or from a specific cohort, isolated in time. The model envisages that neighborhood size as defined by the probabilities of mating, is very small, and dispersal of the bulk of gametes and seeds is very limited. Bradshaw (1972) has stressed this feature of population structure of plants and Levin and Kerster (1971) have computed how apomixis increases, and autogamy decreases neighborhood size. The local population comprises a mosaic of such neighborhoods. The question is how small must neighborhoods be, for various rates of gene flow, in order to explain the observed values of the fixation index without invoking microdifferentiation by selection (Jain and Rai, 1974; Jain, 1975).

An answer to this question must suppose a particular model of subpopulation structure. One such model is the Wright island model which considers subpopulations of effective size N, which receive a portion (m) of genes by migration each generation as a random sample of the entire ensemble. We consider each neighborhood in a plant population surrounded by a large number of adjacent neighborhoods. From Kirby (1975), the expected fixation index of a sample bulked from many neighborhoods (F_{IT}) is,

$$F = (1 - m)^4/[2N - (2N - 2)(1 - m)^2 - (1 - m)^4] \simeq (1 - 4m)/(1 + 4mN) \quad (5)$$

when $m \ll 1$. Table 2 gives some values of F from (5). It is clear that both N and m must be much smaller than commonly envisaged, for the observed values of ΔF. Yet Jain (1976b) and Levin and Kerster (1974) have provided estimates of N from various species in the context of another model, which indicate these low values may indeed be achieved.

TABLE 2

Fixation Index in a Bulked Sample of Neighbourhoods of Effective Size N, Receiving m Migrants per Individual per Generation

	N			
m	10	50	100	200
0.1	0.14	0.03	0.02	0.01
0.05	0.28	0.08	0.04	0.02
0.01	0.69	0.32	0.19	0.11
0.005	0.82	0.49	0.33	0.20

(1F) *Partial Self Pollination*

Brown *et al.* (1975a) considered how, in self compatible species, low levels of self fertilization can increase the observed fixation index. Independent estimates of the mating system are required to establish this possibility, and these have not commonly been made.

(1G) *Negative Heterosis*

Manwell and Baker (1970) have considered this possibility for protein variation between animal subpopulations, but this mechanism has not been studied in plant materials. The models of Jain and Jain (1969) indicate that if frequency dependence of selective values is also involved, stable nontrivial equilibria can be maintained particularly in inbreeders. The result is a positive ΔF.

In summary, then all the above mechanisms have probably contributed to the heterozygosity paradox in outbreeders, with clear evidence implicating 1E, 1D and 1F. As stressed by Bradshaw (1972), natural plant populations are very conspicious in their capacity to fracture into subpopulations each of which can respond adaptively and differentiate selectively over very short distances. The allozyme data so far is entirely in line with this feature. It is interesting to note that studies of outcrossing agricultural populations (Table 1—maize, *Lolium perenne*) tend not to show a lack of heterozygotes. These populations are harvested and replanted at random over generations, so that there is little scope for subpopulation structure to arise.

Let us turn to the other aspect of the heterozygosity paradox, namely why is an apparent heterozygous excess common in inbreeders? Several of the explanations found in the literature will now be considered.

2A. *Heterozygous Advantage for Segments Including the Marker Loci*

This hypothesis has been the one generally favored by the data gatherers in the field (Allard *et al.*, 1968; Marshall and Allard, 1970a; Brown *et al.*, 1974; Rick *et al.*, 1977), and is based on a model originally discussed by Hayman (1953). Let us consider only the case of a diallelic locus, and assume that the two homozygotes have survival selective values s, such that their fitness relative to the heterozygote is $1 - s$. Exact symmetry of fitness values is an unrealistic assumption, made here to simplify the algebraic comparison of the Hayman model with later sections below. More general treatments are given in the references cited. Then the equilibrium fixation index (F) in adults (after selection and prior to mating) is (Workman, 1969)

$$F = \frac{-s + f(2 - s)}{2 - s - fs} \tag{6}$$

where f = the constant amount of inbreeding. In the model of mating systems with constant proportion t of random outcrossing and $(1 - t)$ of selfing.

$$f = (1 - t)(1 + F)/2 \tag{7}$$

We define ΔF as the deviation in the fixation index from that expected at equilibrium for neutral genes, (due here to heterotic selection)

$$\Delta F = F - (1 - t)/(1 + t); \qquad -2/(1 + t) \leqslant \Delta F \leqslant 2t/(1 + t) \tag{8}$$

and

$$f = (1 - t)/(1 + t) + \Delta F(1 - t)/2 \tag{9}$$

The behavior of ΔF with various values of s and t was studied by substituting (9) into (6), and (6) into (8), to obtain the quadratic

$$s(1 - t)(1 + t)^2(\Delta F)^2 - 2[(1 + t)^2 - s(2 - t + t^2)](1 + t)\,\Delta F - 8st = 0 \tag{10}$$

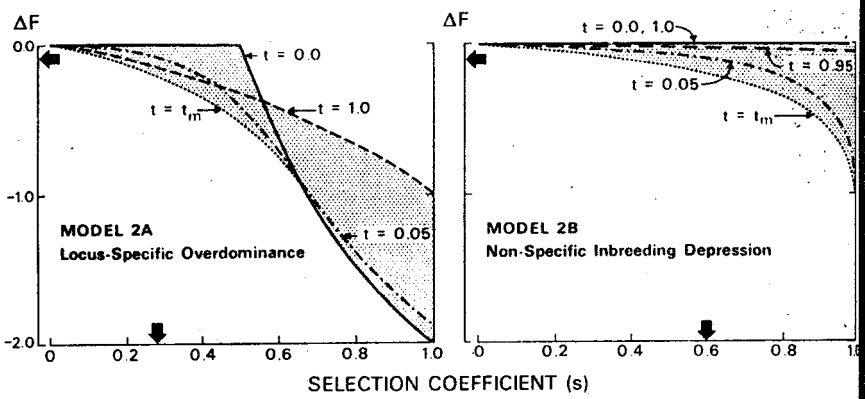

FIG. 1. The effect of locus specific symmetric overdominance (Model 2A) and of nonspecific inbreeding depression (Model 2B), each of selection intensity s, upon the fixation index as measured from that expected at neutral inbreeding equilibrium (ΔF), for various rates of outcrossing (t: $0 < t < 1$) versus selfing ($1 - t$). The feasible region is shaded, and is bounded by the lines for $t = 0$ and/or $t = 1.0$ on the upper edge, and $t = t_M$ on the lower edge, where t_M is that value of t which leads to maximum negative ΔF, for a given value of s.

The values of ΔF for three fixed values of t and varying s are shown graphically in Fig. 1A. The values of t considered are for complete selfing ($t = 0$), predominant selfing ($t = 0.05$), and random mating ($t = 1.0$). A slight amount of selfing (e.g., $t = 0.95$) gives essentially the same curve as for $t = 1.0$. The limiting properties of ΔF are as follows:

$$\lim_{t \to 0} \Delta F = (2 - 4s)/s \qquad 1 > s > 0.5$$
$$= 0 \qquad 0.5 > s > 0$$

$$\lim_{t \to 1} \Delta F = -s/(2 - s)$$

$$\lim_{s \to 1} \Delta F = -2/(1 + t)$$

Just as in the case of gene flow between populations (Spieth, 1974), for outcrossing, "the distinction between absolutely none and almost none is enormous." This is seen in the region $0 < s < 0.5$. Under intense overdominant selection ($s > 0.6$), predominant inbreeding leads to greater net deviations from expected neutral genotypic proportions than outbreeding. When this is taken in conjunction with the point made from (2) above on the sampling variance of F, it is clear why it is experimentally easier to detect deviations from the genotypic proportions expected for neutral genes in polymorphic inbreeders than outbreeders.

Kimura and Ohta (1971) however conclude that overdominance is not effective in self-fertilizing populations. This conclusion rests on their consideration of an inequality they derive which shows that for a stable equilibrium to exist, the proportion of outcrossing must be such that

$$t > \frac{(s_1 - s_2)(1 - 2s_2)}{s_1 + s_2 - 2s_1 s_2} \tag{11}$$

where s_1 and s_2 are the selection coefficients against the two homozygotes ($s_1 > s_2 > 0$). This point is also clear in Hayman's phase diagrams. In general s_1, s_2 must be greater than 0.5, which they claim would be rare. However this inequality (11) essentially controls the *degree of asymmetry* permissible between s_1 and s_2. Hence the question of the effectiveness of overdominance in inbreeders becomes a question of the actual configurations of the $\{s_i\}$ in nature. This is analogous to the problem of maintaining several polymorphic alleles by overdominance (Lewontin *et al.*, 1978). The fact that only severely restricted overdominant configurations are available for maintaining polymorphism does not in itself indicate the likelihood of overdominance compared with other mechanisms of accounting for any observed polymorphism. Furthermore, Eshel (1978) has shown that protected polymorphism may be maintained in two (or more) nonepistatic loci simultaneously by overdominant selection when it could not be maintained in either of them separately.

Two pieces of circumstantial evidence in plant studies argue in favor of a significant role for heterozygote advantage. First many studies have shown that the degree of heterozygote excess varies with the environment (Table 1). In general, large values of ΔF tend to occur in more "extreme" (xeric, marginal) environments. These are often the environments where outcrossing is also lower. Second, preliminary evidence indicates polyploid inbreeders compared with related diploids tend to show at least equivalent, if not greater isozyme diversity but less polymorphism and heterozygous excess (Gottlieb, 1973; Babbel and Wain, 1977; Adams and Allard, 1977; Hart and Langston, 1977), suggesting that these species obtain the putative physiological advantages of diploid heterozygotes (Johnson, 1976), fixed in allopolyploids.

2B. *Heterosis for Outcrosses*

In the above model of Hayman, a basic feature is that selective values are entirely determined by the genotype at the single marker loci. It does not discriminate in fitness of heterozygotes concerning whether they are *newly arisen outcrosses*, or they are produced by *selfing pre-existing heterozygotes*. Cockerham and Rawlings (1967) described a model with contrasting assumptions. Their model considers a single neutral diallelic locus under a mixed mating system [$(1 - t)$ selfing, and t random outcrossing]. Zygotic selection operates such that $(1 - s_i)$ of individuals, which belong to a lineage of i genera-

tions of selfing since their most recent outcrossing, survive to mating. In general equilibrium frequencies are cumbersome functions of the $\{s_i\}$, but when these intensities are positive, there is an excess of heterozygotes.

For comparison with the previous model, we can assume with little loss of generality that the s_i are constant and equal to s ($0 \leqslant s \leqslant 1$). Then the fixation index of the adults is

$$F = \frac{(1-s)(1-t)}{(1+t)-s(1-t)} \tag{12}$$

and the increase over that expected at neutral inbreeding equilibrium is

$$\Delta F = \frac{-2st(1-t)}{(1+t)[1+t-s(1-t)]} \tag{13}$$

For $t = 0$, at equilibrium $\Delta F = 0$ for all s, as selection cannot operate. Similarly $\Delta F = 0$ when $t = 1$. In Fig. 1B, the values of ΔF according to (13) are plotted for the cases of $t = 0.05$, 0.50 and 0.95 for increasing selection intensity s. The value of outcrossing which maximizes ΔF for a given value of s is obtained from partial differentiation of (13) as

$$t_m = [2(1-s)^{1/2} - (1-s)]/(3+s) \tag{14}$$

and these values of ΔF are shown as the curve labelled t_m.

The first point to note is that under this model, the minimum value for F in (12) when $s = 1$ is zero, i.e., the maximum heterozygosity level is $2pq$, the panmictic expectation. In the foregoing Hayman model complete heterozygosity i.e. $F = -1.0$ is possible.

Second, for equivalent values of s, the deviations (ΔF) produced by selection are markedly less in the model 2B than in model 2A. This is especially the case for intense selection.

Conversely, an observed ΔF of say -0.20 requires much less selection to achieve in model 2A (0.33 to 0.53) than in model 2B (0.53 to 1.0).

Finally, the difference between complete Vs predominant inbreeding ($t = 0.0$ vs $t = 0.05$) is most marked in quite different regions of selection. In model 2A, the response to moderate selection ($s = 0.50$ or less) clearly differentiates these two mating systems whereas under model 2B, this occurs for maximal s. Maynard Smith (1977a) has investigated a selection model analogous to model 1B in its impact on polymorphism for a gene promoting selfing. He shows that for constant $s < 0.5$, the selfing habit will increase to fixation. Alternatively, the levels of selection required under model 2B suggested by the field data are of such high intensity, that they would be expected to evolve large changes in the mating system towards reduced selfing.

2C. *Deleterious Mutation/Selection*

Ohta and Cockerham (1974) have considered a development of Model 2B which specifies the nature of selection due to the inbreeding depression more exactly. They considered the effect, on frequencies at a neutral locus, of selection against a partially recessive mutant (recessive mutation frequency v) at a second linked locus (degree of linkage $\lambda = 1 - 2 \times$ recombination fraction). The fitnesses of the genotypes at the second locus are 1, $1 - sh$, $1 - s$. Their "measure of apparent overdominance at the neutral locus," a_Q is in our symbols

$$a_Q = -\Delta F(1 + t)/2t, \tag{15}$$

and is a complex function of (v, t, s, h, and λ). The greatest positive effects on a_Q for varying h is when $h = 0.0$, i.e., a completely dominant mutant. Let us consider this case of complete dominance, and at extremes of linkage ($\lambda = 0$, no linkage and $\lambda = 1$, complete linkage). For $h = 0.0$, and $\lambda = 1$, then $a_Q = v$ and

$$\Delta F = -2vt/(1 + t) \tag{16}$$

This formula shows that the effect on F of such genes is independent of s, and is of the order of the mutation rate. For free recombination

$$\Delta F = -4vt/[1 + t + s(1 - t)][3 + t] \tag{17}$$

which is about half the effect observed for complete linkage. Therefore unless mutation pressures are extraordinarily large, this model seems an unlikely candidate for explaining the values of ΔF found in natural populations. Furthermore this mechanism would be expected to apply to both inbreeders and outbreeders.

2D. *Associative Overdominance*

Sved (1968) investigated the question of how large the apparent selective value would be at a "neutral" diallelic locus for a given amount of linkage disequilibrium (D) with a heterotic locus in a random mating population. [See also Ohta (1973) and Powell (1974)]. In the specially simplified case of symmetric overdominance at the second locus and equal allelic frequencies at the neutral marked locus, the alteration to its fixation index is

$$\Delta F = -16sD^2/(2 - s) \tag{18}$$

More general cases are described in the references cited.

In mixed selfing populations, this is an underestimate of the impact of one locus on another, because correlations in genotypic state arise such that the probability of joint heterozygosity is higher than expected from the single locus frequencies (see Allard *et al.*, 1968 for discussion of earlier literature).

Bennett and Binet (1956) formulated this effect between two loci in the absence of selection.

To demonstrate how heterozygote advantage at one locus (B) can effect F at a second neutral locus (A), we will consider a simplified double diallelic model which assumes

(i) A and B are unlinked.

(ii) Survival selection against homozygotes at B of intensity s is symmetric, which implies the allele frequencies are equal at equilibrium.

(iii) The allele frequencies at the neutral locus are equal.

Point (i) removes any magnification of the effect due to linkage itself. Therefore we examine a minimum bound for the effect. Points (i), (ii) and (iii) greatly simplify the transition equations.

It is sufficient to classify the genotypes into four classes:

g_1 = frequency of adults homozygotes at both loci
g_2 = frequency of adults heterozygous at B, and homozygous at A
g_3 = frequency of adults heterozygous at A, homozygous at B
g_4 = frequency of doubly heterozygous adults.

$$g_1 + g_2 + g_3 + g_4 = 1.$$

The frequencies of adults in the next generation (g_i': $i = 2, 3, 4$) are

$$g_2' = [(1 - t)(2g_2 + g_4) + t]/4\overline{W} \tag{19}$$

$$g_3' = [(1 - t)(2g_3 + g_4) + t](1 - s)/4\overline{W} \tag{20}$$

$$g_4' = [t + g_4(1 - t)]/4\overline{W} \tag{21}$$

where $4\overline{W} = 4 - 2s[1 + (1 - t)(1 - g_2 - g_4)]$.

If $s = 0$, neutrality at both loci obtains, these recursions are easily solved and we have a special case of Bennett and Binet's solutions (see also Workman and Allard, 1962).

$$g_4 = t/(3 + t); \qquad g_3 = g_2 = 2t/(3 + t)(1 + t).$$

If (19) and (21) are added together, at genotypic frequency equilibrium we have

$$X = g_2 + g_4 = \frac{t + (1 - t)X}{2 - s[1 + (1 - t)(1 - X)]} \tag{22}$$

Thus X is the appropriate root of the equation

$$s(1 - t)X^2 + (1 + t - 2s + st)X - t = 0. \tag{23}$$

Since X is the equilibrium frequency of the B heterozygote, $F_B = 1 - 2X$ is related to the quadratic system (6)–(9) above.

The quantity $\bar{W}$ is now defined by this solution of (23)

$$4\bar{W} = 4 - 2s[1 + (1 - t)(1 - X)]. \tag{24}$$

The frequency of heterozygotes at the neutral A locus is

$$Y = g_3 + g_4 = \frac{2t - st + sg_4(1 - t)}{4\bar{W} - 2(1 - s)(1 - t)} \tag{25}$$

where $g_4 = t/[4\bar{W} - (1 - t)]$.

Hence, the fixation index at A is $F_A = 1 - 2Y$ and the change in F_A due to selection at locus B is

$$\Delta F_A = 1 - 2Y - (1 - t)/(1 + t). \tag{26}$$

TABLE 3

Alteration to Fixation Index (ΔF) at a Neutral Locus from Symmetrical Overdominant Selection Operating at Another Unlinked Locus

Selection intensity (s)	Outcrossing rate (t)					
	0.05	0.10	0.20	0.30	0.50	0.90
0.1	−0.006	−0.010	−0.012	−0.012	−0.009	−0.001
0.3	−0.023	−0.034	−0.041	−0.038	−0.022	−0.004
0.5	−0.043	−0.058	−0.064	−0.059	−0.039	−0.006
0.6	−0.041	−0.058	−0.066	−0.061	−0.042	−0.007
0.7	−0.031	−0.048	−0.059	−0.056	−0.040	−0.007
0.9	−0.009	−0.016	−0.022	−0.024	−0.019	−0.004

Values of ΔF_A, the alteration to the fixation index at a neutral locus, from symmetrical heterozygous advantage at another *freely recombining* locus are given in Table 3, for equilibrium under particular values of selection and outcrossing. As such, they represent minimum values for the Bennett and Binet effect extended to one symmetrical, overdominant locus. The bias would be increased by linkage (Allard *et al.*, 1968, Fig. 7). These figures indicate that a substantial negative bias to F estimate when selection is intense ($s \cong 0.6$) and under moderate outcrossing ($t \cong 0.25$). Strobeck (1978) has obtained the equilibria for the above two locus model (one neutral, the other heterotic), with an arbitrary degree of linkage between the loci.

A general formulation of the single generation hitchhiking effect under the mixed mating system which takes into account gametic disequilibrium, zygote associations, linkage intensity and their interaction has yet to be made. It is

likely that all three phenomena will have a reinforcing interaction with regard to the effect of an overdominant locus on genotype distribution at a neutral marker locus, and this could contribute substantially to the heterozygosity paradox in inbreeders. However the domains of s and t in which these forces operate, and the accompanying effect of this selective mode on genes modifying t are yet to be specified.

2E. *Difference in Gene Frequency Between Male and Female Gametophytic Cohorts*

Robertson (1965) showed that in outbreeding populations, differences between sexes in the frequencies of alleles which might arise randomly from a small number of adults would generate an excess of heterozygotes over Hardy Weinberg expectations in the progeny of the sampled parents. Purser (1966) showed how any variation in fertility with genotype in either or both sexes, would generate such an excess by a similar mechanism. In *Lupinus nanus*, Horovitz and Harding (1972) found evidence of differential male outcrossing rates for different genotypes. For inbreeding populations, Harding (1975) considered a model of reciprocal gametophytic selection and pointed out that the excess of heterozygotes generated would simulate the effects of sporophytic heterozygote advantage when in fact the heterozygotes had no advantage at all.

In highly inbreeding populations, even when the total number of mature plants is *large*, the number of pollen grains involved in outcrosses might be very small. In samples from such populations which could include extremely few outcrossed male gametophytes, there might appear to be scope for the Robertson bias to operate. To investigate this we consider a general transition formula for the frequency of heterozygotes in the seeds (H'), in which two alleles occurred with frequency r, $\bar{r}$. Where the frequency of maternal heterozygotes is H and maternal gene frequencies are q, $\bar{q}$ and pollen frequencies p, $\bar{p}$ with outcrossing rate t, H' is given by

$$H' = t(p\bar{q} + q\bar{p}) + H(1 - t)/2$$

and (27)

$$r = (1 - t)q + t(p + q)/2$$

Since $H' = 2r\bar{r}(1 - F')$ and $H = 2q\bar{q}(1 - F)$

$$\begin{aligned} \Delta F &= F' - F \\ &= [(1 - F)(2r\bar{r} - q\bar{q}(1 - t)) - t(p\bar{q} + q\bar{p})]/2r\bar{r} \\ &= \{(1 - F)[(1 - t)\,q\bar{q} - t^2(\bar{p} - \bar{q})^2/2] - Ft(p\bar{q} + \bar{p}q)\}/2r\bar{r} \end{aligned} \quad (28)$$

When $F = 0$ and $t = 1$ this quantity is negative which is Robertson's result

However, the bias from inbreeding equilibrium $F[=(1-t)/(1+t)]$ is:

$$\Delta F = \frac{(1-t)(q-p)(1-2q) - t^2(p-q)^2}{2r\bar{r}(1+t)/t} \tag{29}$$

which can be either positive or negative provided ($t < 1$). Therefore differences between effective parental gene frequencies due to sampling, or variation in pollen gene frequencies away from maternal frequencies, will not always lead to an apparent excess of heterozygous progeny over neutral inbreeding equilibrium expectations.

2F. *Finite Population Size*

Kirby (1975) reviews earlier literature on the frequencies of heterozygotes in small populations and develops an expression for the effect on the fixation index of small population size (F_{IS}). Biases in the estimates of F arise both from finite sampling (Levene, 1949) and from differences in gene frequency between male and female parents above.

For moderate values of the variance effective population number ($N > 20$)

$$\Delta F \cong -1/N.$$

In monoecious populations, if there is no difference in gene frequency between male and female gametes then ΔF becomes $-1/(2N-1)$ (Cockerham, 1973). These formulae indicate that these effects play a minor role in the reported studies of inbreeding populations. However this conclusion is at variance with those of Jain and Rai (1974) and Jain (1975) who found that in the island model of subdivision, ΔF may be positive or negative and in amounts more than 0.10 for moderate subpopulation sizes of 100.

2G. *Temporal Fluctuation in Outcrossing*

Nei (1975) discussed the possibility that variation in outcrossing rate in time might be the source of negative ΔF. This appears because in any one generation following one with an unusual burst of outcrossing, the frequency of heterozygotes can be much higher than the equilibrium value under constant outcrossing. Because "random mating restores the Hardy-Weinberg equilibrium in one generation, while selfing reduces the frequency of heterozygotes only by a half each generation" one is led to enquire when temporal fluctuation in outcrossing would consistently lead to a bias in heterozygosis expected compared with that under a constant level of outcrossing but the same mean. Jain and Marshall (1968) examined by simulation the effect of stochastic outcrossing on the conditions for the existence of selectively balanced polymorphism. They found that under a wide variety of probability distributions for t, there was very little change to the critical bounds of the selection

coefficients. However, this does not answer the above question regarding heterozygous frequencies.

Consider the transition equation for F for a neutral polymorphism

$$F_j = (1 - t)(1 + F_{j-1})/2$$

where F_j is the fixation index at the jth generation. If we put $X = (1 - t)/2$ and consider X a random variable with distribution $f(X)$

$$\begin{aligned} F_j &= X_j(1 + F_{j-1}) \\ &= X_j[1 + X_{j-1}(1 + F_{j-2})] \\ &= X_j + X_jX_{j-1} + X_jX_{j-1}X_{j-2} + \cdots \end{aligned} \tag{30}$$

If all the X_j are constant we have $E[F_j] = (1 - t)/(1 + t)$. Otherwise

$$E[F_j] = E[X_j] + E[X_jX_{j-1}] + \cdots$$

If X_j is independent of X_{j-1}, and there is no autocorrelation of outcrossing rates in time

$$\begin{aligned} E[X_jX_{j-1}] &= \{E[X_j]\}^2 \\ E[F] &= \sum_{i=1}^{\infty} (E[X_j])^i \\ &= (1 - t)/(1 + t) \end{aligned} \tag{31}$$

Therefore when outcrossing rates fluctuate randomly in time, irrespective of the probability distribution of t_j, but without autocorrelation between generations, there is no consistent bias to the estimated level of heterozygotes. This point is confirmed in simulation studies reported by Allard *et al.* (1968). Fluctuation of outcrossing might however contribute to a reduced estimate of F in any one particular study, but not to an overall trend.

Unfortunately, the conclusion is entirely different for the effect of stochastic variation of outcrossing on the expected inbreeding coefficient (F_e), estimated from the current level of outcrossing. As is shown in the next section (2H) in another context, the average of several values of F_e, each pertaining to a different value of t, is higher than the single value of F_e computed from the observed mean of those same values of t. Therefore fluctuation in outcrossing does lead to a biased estimate of ΔF towards negative values; not because estimates of heterozygosity or the fixation index (F) are biased, but because estimates of the effect of inbreeding (F_e) are biased. The bias can be reduced by making several temporally or spatially independent estimates of outcrossing, and using the observed mean in a test of ΔF. In so far as this bias is a technical problem, applying to studies of both inbreeding and outbreeding plant species, we will not consider it further as a major factor in the heterozygosity paradox.

2H. *Spatial Variation in Outcrossing*

Another possible source of bias arises when the sample is actually drawn from a mixture of K sub-populations each of which are at equilibrium for a different level of outcrossing. Suppose that t_i *irrespective of genotype* is the outcrossing rate for the ith sub-population which has achieved local neutral inbreeding equilibrium for this rate. This model might obtain when sub-populations vary in humidity or exposure to wind, or where animal activity is highly localized and variable in patches. Further suppose for simplicity that each sub-population contributes $1/K$ to the sample, and has the same gene frequencies (p, q).

The frequency of heterozygotes in the sample is,

$$\begin{aligned} H &= \frac{4pq}{K} \sum_{i=1} t_i/(1 + t_i) \\ &= \frac{4pq}{K} \sum_{i=1} (t_i - t_i^2 + t_i^3 - \cdots) \end{aligned} \tag{32}$$

For *small* t_i ,

$$H \cong 4pq[\bar{t} - (\bar{t})^2 - \sigma_t^2]$$

where σ_t^2 is the variance of outcrossing. Therefore the observed fixation index would be

$$F \cong 1 - 2[\bar{t} - (\bar{t})^2 - \sigma_t^2]. \tag{33}$$

If the mean outcrossing frequency $(\bar{t})$ were separately estimated, the expected fixation index would be,

$$\begin{aligned} F_e &= (1 - \bar{t})/(1 + \bar{t}) \\ &\cong 1 - 2[\bar{t} - (\bar{t})^2] \end{aligned}$$

and ΔF would be $2\sigma_t^2$ and positive. Thus, the bias arising in this model would explain overall heterozygous deficiencies rather than excesses.

In general, it appears that the observed order of magnitude of heterozygous excess in inbreeders is such that mechanisms 2B, 2C, 2E, 2F, 2G and 2H contribute only marginally to the phenomenon, and heterozygous advantage at the loci themselves, or other loci closely linked and in strong disequilibrium with them is indicated. As Workman (1969) pointed out, observed genotypic proportions in any population represent the net effect of several phenomena each of which gives rise to either positive or negative ΔF. Therefore, further investigation is generally required to support conclusions derived from F statistics alone.

B. Extent of Multilocus Associations

During the entire electrophoretic decade, it has been stressed by many authors that analyses of complex polymorphic data, at the multilocus level would provide a greater insight into the forces controlling enzyme polymorphism in natural populations (e.g., Workman, 1969; Franklin and Lewontin, 1970; Allard and Kahler, 1971; Lewontin, 1973, 1974; Allard, 1975). Yet several problems continue to bedevil the study of multilocus dynamics. At the experimental level, there is the difficulty of finding several loci sufficiently closely linked ("five loci within 5 recombination units"; Lewontin, 1973) in outbreeding organisms, and the requirement of adequate sample size (Brown, 1975). In estimation, the optimal parametric measures of multilocus associations are unknown. In interpretation, it is clear from theory that there is no one to one correspondence between the occurrence of disequilibria, and the necessary operation of either single locus or epistatic selection (see Thomson, 1977 and Hedrick *et al.*, 1978 for discussion). Furthermore the rates of approach to equilibrium expectations of association and descent measures can be extremely slow (Weir and Cockerham, 1973, Weir *et al.*, 1974).

Nevertheless, studies of allozyme disequilibria in plant populations, admittedly all too few, have demonstrated one general conclusion namely that multilocus associations are a much more obvious feature of predominantly selfing species, than of outcrossing species (Allard, 1975). For example in two *Zea mays* populations, the only significant disequilibrium detected arose between two closely linked loci as an effect of small population size at foundation and during selection of parents (Brown and Allard, 1971). In populations of the outbreeding trees *Eucalyptus obliqua*, and *E. pauciflora* (Brown *et al.*, 1975a; Phillips and Brown, 1976) two-locus disequilibrium values were not statistically significant. The observed average absolute standardized disequilibria were 0.24 and 0.29 respectively. When gene frequencies are asymmetric this intensity of association between unlinked genes easily arises by chance even in moderate sample sizes. No genotypic associations at pairs of four loci were found in *Silene maritima* (Baker *et al.*, 1975). In *Lolium multiflorum*, Mitton *et al.* (1978) have evidence of association at the 3-locus level, without associations apparent at the 3 two-locus comparisons. In general, the sample sizes required to detect three-locus effects are minimized when there are no two-locus disequilibria (Brown, 1975).

In inbreeding species, intense disequilibria have been found in natural populations of *A. barbata* (Allard *et al.*, 1972), *Hordeum spontaneum* (Brown *et al.*, 1977) and in cultivated populations of barley, *H. vulgare* (Clegg *et al.*, 1972). This characteristic of predominantly inbreeding populations indicates that the role of mechanism 2D above in explaining heterozygote excess deserves special emphasis.

The pattern in *A. barbata* is particularly striking. Polymorphic populations

in California consist largely of particular 5-locus gametic types which also differentiate the more and less xeric regions of the state. Clearly, the close inbreeding assists in preserving these associations, even between the unlinked loci. The authors postulate that not only are the two multiple homozygotes specifically favored (a multiniche model) but also the complexes interact favorably in multiple heterozygotes. For this hypothesis, the disequilibria arise both from population subdivision (Nei and Li, 1973) and from epistatic heterozygote advantage.

The nature of linkage disequilibrium at four esterase loci in composite crosses II and V of barley has been examined on two levels (i) the relationship between joint gametic frequencies and their single locus marginal frequencies (Clegg *et al.*, 1972; Smouse, 1974; Weir *et al.*, 1974); and (ii) estimates of two-locus zygotic selective coefficients (Weir *et al.*, 1972). In the first study, Clegg *et al.* (1972) showed using chi-square statistics that correlations in allelic state intensified during the evolution of composites. These correlations were expressed both by closely linked, and to a lesser extent by the freely recombining sets of loci. Favored associations were defined as those showing excessive discrepancies in frequency compared with frequency predicted by the product of their single-locus marginal frequencies. In some cases the absolute frequency of these "favored" types is actually declining, indicating that the effect could in fact be due to directional selection at these or other linked loci. However the authors argue against the hitchhiking hypothesis mainly on the grounds that the same two complementary types were favored in the two independent composites. This argument is weakened by the fact that these two composites had 11 parental lines (about 30%) in common.

Smouse (1974) reanalyzed these data by partitioning the two, three and four locus effects using a logarithmic model. He concluded that most of the multiple locus disequilibrium is accounted for by two-locus effects.

Weir *et al.* (1974) computed the two-locus associations in composite cross V after taking into account its initial composition and evaluating the two-locus the descent measures of Weir and Cockerham (1973). They found that the direction of deviation from the expectation of the product of the marginal gene frequencies was largely governed by the direction of initial disequilibria at synthesis, but the degree of association increased with time, especially for the tightly linked esterase loci A, B and C. Selection either direct or indirect, was undoubtedly the major force determining which of pre-existing associations were to increase. (Linkage was too tight to allow any major production of recombinants before multiple heterozygosity was drastically reduced). This conclusion is supported by two-locus zygotic fitness estimates (Weir *et al.*, 1972) derived from sequences of generations. Double homozygotes at pairs of the four esterase loci show highly significant differences in fitness. The sampling errors on fitness estimates of single and double heterozygotes, despite the large sample sizes, were too large for these estimates to differ significantly

from 1.0 but taken overall, they indicated a 52% superiority over homozygotes. In general the two-locus fitness estimates were not predictable from their single locus values. However their proposed demonstration of particular selection effects attributable to the A locus (the internal locus of the closely linked triad B-A-C), would require the assumption that the rest of the genetic background is randomized with respect to each BAC triad. The evidence discussed above clearly shows this cannot be assumed.

The drastic difference between outbreeders and inbreeders in the occurrence of linkage disequilibria merits further comment. In theory, the restriction on recombination due to predominant selfing does not guarantee the evolution of gametic associations between neutral genes. In large, equilibrium populations such associations should disappear. (This is not true of zygotic associations—see 2D above). However this mating system does greatly increase the scope for retention of associations developed by directional or epistatic selection, or by sampling effects in subdivided populations. Similarly associations with structural hybridity (Levy and Winterheimer, 1977) may be anticipated. These mechanisms assist in the congealment of the genome (see Maynard Smith, 1977b for review). Conversely it is therefore relevant to develop models of the evolutionary modification of the mating system which take into account not only hybridity (2B above) but also the selective dynamics of multilocus associations. Finally, the empirically established differences between outbreeders and inbreeders (or chromosomally polymorphic populations) indicates that when disequilibria are present, they can be found experimentally. This result does not support the suggestion of Zouros *et al.* (1976) and Langley *et al.* (1977), that the lack of disequilibria in outbreeders is due to the lack of resolution by electrophoresis—"hidden" disequilibria between "hidden" alleles.

C. Extent of Microgeographic Differentiation

There is a large amount of evidence for morphological and physiological attributes indicating the extent to which a plant population can differentiate markedly, despite considerable gene flow (Jain and Bradshaw, 1966; Stern and Roche, 1974). Such differentiation can take place over remarkably short distances, and is adaptive (Bradshaw, 1972). However, there have been so far relatively few studies of this phenomenon at the level of enzyme polymorphisms. The results of these studies are summarized in Table 4, using measures of genetic diversity as proposed by Nei (1973). Thus, where p_{ij} is the frequency of the ith allele in the jth subpopulation ($j = 1, 2, \ldots, k$), the within subpopulation diversity is computed as

$$H_j = 1 - \sum_i p_{ij}^2/k$$

and

$$\bar{H} = \sum_j H_j/k$$

Assuming equal contributions, the overall diversity in the study is

$$H_T = 1 - \sum_i \bar{p}_{ij}^2$$

$$= \bar{H} + D_{ST}$$

TABLE 4

Studies of Microgeographic Differentation in Plants

Species	Variable Loci	Sub Popns.	$\bar{H}$	$D_{ST}/\bar{H}$ (%)	Authors
Inbreeders					
1. ***Avena barbata***	5	7	0.27	35	Hamrick and Allard (1972)
2. *Avena barbata*	5	17	0.27	50	Allard *et al.* (1972)
	4	20	0.29	15	
3. *Avena fatua*	+24(?)	16	0.15	59	Jain and Rai (1974)
4. *Bromus mollis*	6	10	0.21	3	Brown *et al.* (1974)
Outbreeders					
5. *Lolium multiflorum*	4	4	0.26	1	Mitton *et al.* (1978)
	3	4	0.31	1	
6. *Silene maritima*	3	6	0.32	4	Baker *et al.* (1974)
7. *Liatris cylindracea*	15	66	0.09	7	Schaal (1975)
8. *Pinus ponderosa*	1	8	0.33	3	Mitton *et al.* (1977)

+ Data from this study are for 24 isozyme band positions, the mean % polymorphic bands, and the coefficient of variation between colonies.

The relation between this measure of differentiation (D_{ST}) and Wright's F_{ST} (Wright, 1965) is

$$D_{ST}/\bar{H} = F_{ST}/(1 - F_{ST})$$

The statistics H and D_{ST} are analogous to the Simpson measures of α and β species diversity (Whittaker, 1972).

In all these studies except (5), the differences in gene frequency between subpopulations were statistically significant, with evidence of partial relationship with environmental factors in studies (1, 2, 4, 7, 8). Also in *Liatris cylindracea* and *S. maritima*, there is evidence of a positive relationship between spatial distance and genetic distance (Schaal, 1974 and Baker *et al.*, 1975), indicating the importance of limited gene dispersal. The increase in

heterozygosity at variable loci in a hypothetical, randomly mated bulk of closely adjacent subpopulations is about 4% in outbreeders and probably higher in inbreeders. This estimate may be contrasted with the corresponding figure between different Carribbean island populations of *Drosophila willistoni* (Brown *et al.*, 1975b) of 1.6%. The contrast indicates the extent to which plant populations are differentiated locally. The interactions between selection and gene flow in parapatric or sympatric multiniche situations is receiving much attention from theoreticians recently (see reviews of Felsenstein (1976) and Hedrick *et al.* (1976)). To some extent, more recent theory has met Bradshaw's (1972) criticisms for relevance to plant populations. These were the need to consider models with strong selection (e.g., Nagylaki, 1976) and limited migration in multiniche situations. However, more attention has been paid to conditions for protected polymorphism (Christiansen and Feldman, 1975; Karlin, 1975) than to predicting the extent of differentiation. An example of the latter emphasis is the simulation study of Dickinson and Antonovics (1973). They used a diallelic model of a mosaic patchy environment and stochastic selection to determine the conditions under which the correlation between genotype and environment, and the level of heterozygosity were increased, despite unlimited seed dispersal.

In the abovementioned reviews, there has been comparison on the relative power of temporal as opposed to spatial variation in fitness for maintaining polymorphism. A basic finding in the haploid case, is that temporal variation in fitness has no tendency to maintain polymorphism. To the extent that predominant inbreeders, as opposed to outbreeders, can be considered populations of "haploids," one might expect to find much less gene frequency variation between generations (a D_{ST} computed over years) within the set of variable loci in inbreeding populations. No data are presently available to test this. In the case of localized spatial differentiation, the existence of pockets of adaptation is indicated. The level of migration plays a crucial role in the theory of such pockets (see Felsenstein, 1976). Presumably it is the effect of mating system on this parameter which accounts for the more intense differentiation observed in most selfing populations.

D. Geographic Variation

Several studies of allozyme variation in plants have examined geographic variation between populations. These results are summarized in Table 5, using the same statistics as in Table 4. Other reviewers (Gottlieb, 1977a and Hamrick, 1978) have used estimates of Nei's genetic identity or distance for this purpose. These measures assume that a *random* set of loci are examined and the data of all these loci including the *invariant* ones are included. As such they indicate the extent to which populations have diverged. In Table 5, the invariant loci are excluded from consideration and the emphasis is thus

TABLE 5

Estimates of Geographic Differentation for Allozymes in Plants

Species	Number of Variable loci	Number of Pop'ns	$\bar{H}$	$D_{ST}/\bar{H}$ (%)	Authors
Outbreeders					
Clarkia biloba	5	2	0.270	14	Gottlieb (1974)
Clarkia lingulata	5	2	0.278	23	
Clarkia rubicunda	6	4	0.239	14	Gottlieb (1973a)
Lupinus subcarnosus	5	8	0.170	14	Babbel and Selander (1974)
Lupinus texensis	5	10	0.497	5.3	
Phlox drummondii	7	73	0.209	26	Levin (1977)
Phlox roemariana	4	15	0.278	35	Levin (1978)
Stephanomeria exigua	8	11	0.297	11	Gottlieb (1975)
Zea mays -Mexico	1	51	0.604	39	Stuber *et al.* (1977)
Zea mays -Guatemala	1	38	0.621	25	
H. scabiosaeus	5	14	0.306	10	Babbel and Selander (1974)
H. artemisiaefolius	5	12	0.311	19	
Lycopodium lucidulum	5	16	0.145	53	Levin and Crepet (1974)
Eucalyptus obliqua	3	4	0.370	32	Brown *et al.* (1975)
Eucalyptus pauciflora	7	3	0.279	2.2	Phillips and Brown (1977)
Ficus carica	2	4	0.530	2.3	Valizadeh (1977)
Pinus sylvestris	3	3	0.331	14	Rudin *et al.* (1974)
Picea abies	4	8	0.424	4.5	Bergmann (1974)
Picea abies	6	10	0.403	4.7	Tigerstedt (1974)
Picea abies	4	8	0.346	2.4	Lundkvist and Rubin (1977)
Inbreeders					
Avena barbata	5	16	0.071	340	Clegg and Allard (1972)
Avena barbata	9+	31	0.239	120	Kahler *et al.* (1978)
Avena fatua	6	5	0.183	101	Clegg (1972)
Hordeum spontaneum	25	28	0.098	98	Nevo *et al.* (1978)
Hordeum jubatum	5	3	0.231	21	Babbel and Wain (1977)
Phlox cuspidata	6	43	0.044	91	Levin (1978)
Lycopersicon pimpinellifolium	11	43	0.142	80	Rick *et al.* (1977)
Oenothera biennis Cook	1	16	0.094	294	Levin (1975)
Oenothera biennis Illinois	4	28	0.220	113	
Tragopogon porrifolius	2	3	0.147	94	Roose and Gottlieb (1976)
Tragopogon dubius	5	6	0.117	98	
Tragopogon miscellus	4	6	0.022	5	
Tragopogon mirus	5	8	0.027	550	
+ enzyme assays					

Summary

Outbreeders	Average	0.345	17
	Weighted average	0.288	20
Inbreeders	Average	0.126	118
	Weighted average	0.127	106

on partitioning the variation detected. Comparisons between the results of various authors will thus be less affected by the randomness and completeness properties required of the I measures. Average and weighted averages for the outbreeding and predominantly inbreeding species are given, where the weighted average D_{ST} is obtained before dividing by the weighted average $\bar{H}$. By these criteria, individual populations of outbreeders are about twice as diverse per locus on average. However, inbreeding plant species exhibit far greater diversity between populations than outbreeders—about five times greater relative to their intrapopulation variation; or about twice the level in absolute (D_{ST}) terms. Populations of both groups are much more highly differentiated than in typically highly vagile animals.

Three general approaches have been taken by authors to allozyme differentiation between plant populations. Many of the reports have merely demonstrated that significant differentiation between populations is present. A second step is to search for and describe patterns of variation where the testing may be fairly *ad hoc*. More recently there has been a trend to subject the total data to biometric analysis to demonstrate patterns. We consider briefly the patterns reported, and then the results of more quantitative approaches.

Clegg and Allard (1972) reported a striking case of geographic differentiation for *A. barbata* populations in California. In the more xeric valley region of the state, populations were monomorphic for a single five-locus genotype. In contrast the cooler, moister and more mountainous area supported populations which were frequently polymorphic and predominantly with different alleles at these loci. Levin (1975a) found a similar contrast in level of diversity between populations of *Oe. biennis* in more northerly Cook County, as opposed to the remainder of Illinois. At another extreme, is the striking endemism of 12 of the 14 variant alleles in *Lycopersicon cheesmanii*, each being restricted to one or two Galapagos Islands (Rick and Fobes, 1975). Babbel and Selander (1974) compared the diversity levels in the geographically and edaphically more restricted *Lupinus subcarnosus*, with *L. texensis* and found it had less genetic variation both within and between populations.

Stuber *et al.* (1977) studied the allelic distribution of the polyallelic β-glucosidase locus in races of *Z. mays* and noted allelic occurrence varied with altitude. Bergmann (1975) demonstrated a latitudinal cline for acid phosphatase in two species of conifer. Rick *et al.* (1977) sampled 43 populations of *Lycopersicon pimpinellifolium* along the west coast of Equador and Peru. They found several clines for allele frequency, genetic diversity, and outcrossing rate. They classified these into (i) simple regional clines, (ii) single peaked clines, and (iii) double peaked clines. This study exhibits a very high degree of pattern. Tigerstedt (1974) found latitudinal clines in *Picea abies* evident for 2 esterase loci, but lacking for 2 peptidase loci. Levy and Levin (1975) reported significant latitudinal or longitudinal clines for four loci in some members of the *Oe. biennis* complex.

An extended consideration of the biometric procedures applicable to the whole set of data is beyond the scope of this review because this is a highly developed field (see for example Thorpe, 1976 for recent review and references), yet one in which few studies of plant allozymes have been made. Two kinds of questions can be asked: (i) to what extent do the different populations show a geographic pattern in their differences of allozyme frequencies (single or multiple loci), or genetic diversity?; and (ii) are there associations between geographically varying environmental parameters and genetic variation?

With regard to geographic patterns, Levins (1977) used the test of Royaltey *et al.* (1975) for allozyme data in *Phlox drummondii*. He found that the intra-population genetic diversity and certain alleles at 3 of 7 variable loci each exhibited non random spatial pattern. However the test failed to reveal any departure from non random spatial patterns in *P. roemariana* and *P. cuspidata* (Levin, 1978). Yet these species are highly differentiated (Table 5). Similarly, Nevo *et al.* (1978) found no correlation in Israel populations of *H. spontaneum* between the mean genetic distance of each population from all others, and the analogous mean geographic distance. In general it would appear that geographic proximity can be a poor predictor of genetic similarity.

Associations with environmental variables have been studied in *A. barbata* (Kahler *et al.*, 1978) and *H. spontaneum* (Nevo *et al.*, 1978) by step-wise multiple regression. Linear associations were found for diversity measures, as well as allozyme frequencies at certain loci in both studies. Diversity in *H. spontaneum* exhibited a marked curvilinear association with rainfall, reminiscent of the single-peaked clines of Rick *et al.* (1977).

It is clear that the associations found in studies of this kind are descriptive in character, rather than deterministic. Such associations probably arise in many ways, and do not establish direct control between either spatial or environmental variables and genetic variation. They are useful summaries of data, and with respect to the causes of variation, are hypothesis generating rather than discriminating. The finding of contrasting patterns of variation (e.g., clines Vs intense local differentiation Vs uniform frequencies) at different loci in the same material has been argued as suggestive of a role for selection at some of the loci (Christiansen and Frydenberg, 1974). However attempts at a formalized test of contrasting patterns have met considerable criticism (Ewens and Feldman, 1975).

E. Components of Selection and Genetic Demography

The need for a functional and ecological concept of fitness and of selective differences between genotypes has recently led experimentalists to investigate components of the selection process, on the one hand, and genetic demography on the other. Selection component analysis attempts to estimate coefficients pertaining to specific phases of the life cycle, or specific ages or physiological

states of the organism. The life cycle phases can be broadly grouped (see Clegg, Allard and Kahler, 1978a) into viability components (dormancy, germination, establishment survival to maturity, longevity), and fertility components (age of flowering, pollen and ovule production, segregation distortion, gametophytic selection, pollination and seed development). The estimation of such components requires either (i) the determination of genotypic frequencies in at least two different stages of the life cycle (either directly, or indirectly through mother-child combinations) separated in time; or (ii) the determination of a demographic attribute (e.g., viability, seed number, etc.) for each genotypic class.

"Demography, in its broadest sense, is simply the statistical study of human populations.... In its more usual, narrower sense, demography refers to the study of mortality and fertility as a function of age.... Genetic demography is the extension of population genetics to take account of the life table information on populations" (from Cavalli-Sforza and Bodmer, 1971). It is clear that in the current literature, these terms are in the process of acquisition by plant population biologists via the zoologists, and acquiring new and varying meanings. Harper (Harper and White, 1974, and Harper, 1977) has exhaustively reviewed the field of plant demography and demonstrated its parametric richness. The basic notion in ecological demographic studies is *numbers* (or density) and a major task of the demographer is "to measure, describe and explain changes in the numbers that compose a population." (Harper and White, 1974). Experiments in genetic demography can overlap those in selection component analysis but no study of allozyme variations in plants has yet really encompassed both fields.

The first kind of selection component data is exemplified in plants by the work of Clegg, Allard and Kahler (Clegg and Allard, 1973; Kahler *et al.*, 1975; Clegg *et al.*, 1978b and reviewed in Allard *et al.*, 1977 and Clegg *et al.*, 1978a). Their first study of three esterase polymorphisms in a natural population of *A. barbata* revealed a strong viability component in favor of heterozygotes, and little evidence of fertility variation between genotypes. Their much more detailed study is of the esterase polymorphisms in composite cross V of barley (*H. vulgare*). Remnant seed of three generations was grown in the same environment, and selection components were estimated for viability and fecundity, and within the pollen pool. Evidence of strong selection was found in all three phases, at the single locus level, and at the three locus level. Complementary effects were common (i.e., a genotype being favored in viability but disfavored in fecundity, or an allele being favored in the male gametophytic phase but disfavored among ovules). Such complementary effects are difficult to interpret biologically because they can in part arise from statistical negative correlations between different components which are not estimated in separate experiments. In addition, because this population is highly self pollinated, the appropriate models for temporarily fluctuating selection would be those for haploids. A

noted above, such models indicate that this type of selection alone cannot maintain polymorphism. Another feature of their data was the inconsistent performances of several genotypes in the different generations, despite the testing in a common environment, presumably because the genetic background had changed. Furthermore, multilocus estimates bore little relation to their single locus components. These results complicate the notion of marginal fitness attributable to marked segments, in these materials. Without mortality and fecundity schedules it is impossible to tell what fraction of the variance in demographic characters was absorbed in differential selection.

There are many more examples of the second type of selective component data. With respect to genotype specific *germination* rates, the alcohol dehydrogenase allele associated with greater enzymatic activity in a *Bromus mollis* population, also showed speedier germination at cool temperatures (Brown *et al.*, 1976). In two of three populations of *E. pauciflora*, estimates of "apparent outcrossing" obtained from allozyme markers expressed in seeds, were significantly lower than estimates of the same parameter made from markers expressed in young seedlings (Phillips and Brown, 1977). This indicated that inbred seeds were disfavored in early germination and establishment.

With respect to *survival* properties, the *Adh* polymorphism has been studied in several species. In *Z. mays* (Marshall *et al.*, 1973) and in *B. mollis* (Brown *et al.*, 1976) the allozyme associated with lower enzymatic activity showed a higher growth rate than its alternative, when tested under waterlogged conditions. In *Helianthus annuus* Torres *et al.* (1977) found an *Adh* allele which showed a shorter duration of enzyme activity during seed development, was more frequent in waterlogged sites. Gene frequencies at an *Adh* locus were the only set among 9 polymorphic loci to change markedly during the course of reciprocal recurrent selection for yield in a maize population (Brown and Allard, 1971). Hamrick and Allard (1975) report differences in maturity time, height and tiller number between two five-locus genotypes of *A. barbata*. Gottlieb (1977) studied allozyme frequencies at 7 loci in individuals of contrasting sizes in a population of *Stephanomeria exigua*. He found no significant differences in genotype frequencies between sets of large Vs small individuals, and their progeny had similar size and growth rate on testing. This result appears to contrast with the current emphasis of all other studies here mentioned. It is clearly too early to conclude that the common ecological practice of ignoring the genotypes of individual plants when accounting for the reproductive activity of a population, even when individuals make very different contributions to the seed pool, is validated. With respect to *fecundity* differences, Schaal and Levin (1976) found in *L. cylindracea*, that heterozygous seedlings tended to flower earlier in the greenhouse. Linhart *et al.* (1978) found an association between female cone production and peroxidase or esterase genotype in *Pinus ponderosa*.

The main drawback to several of the above studies as examples of genotype

specific fitness is that, on their own, the studies are evidence of differential adaptedness of the variants. The extent to which these differences are translated into selective coefficients depends at least on phenotypic plasticity and environmental variation, the duration of the relevant phase and population density (Brown *et al.*, 1976). Thus several of these studies lack the power of the multiple genotypic frequency determinations (in time or space) of Clegg *et al.* to show that selective transformation is actually taking place in natural populations. However, they could provide greater physiological insight into selective differences.

One of the best plant population studies to integrate demographic and genetic variables is that of Schaal and Levin (1976) in the perennial self incompatible herb, *L. cylindracea*. The basic demographic variable was the age distribution in which a deficiency of young plants was noted. Cohorts of increasing age were found to have steadily increasing heterozygosity levels, and decreasing differentiation between quadrats. Other positive correlations between heterozygosity and biomass or reproductive potential may have arisen from the older larger corms. From these data, Schaal and Levin (1976) argue that "the case for heterozygote advantage in *Liatris cylindracea* is compelling." However the most puzzling feature about this conclusion is that overall, the population exhibited a remarkable average *deficit* of heterozygotes of about 50% compared with Hardy Weinberg expectations. The authors suggested that this might arise from either inbreeding, or restricted neighborhood size. Clegg *et al.* (1978a) considered these suggestions and computed that either the effective self fertilisation would have to be 84%; or the neighborhood size was $N = 1.84$. Clearly neither of these factors alone could account for the data. Both might operate to bring about part of the observed deficit. However to invoke heterozygote advantage as well, amounts to saying that inbreeding and neighbor effects must be even more severe than need be for neutral genes.

The main ambiguity arises in the data because genotype census is only available at one point in time. Therefore "the F statistics can also be viewed retrospectively" (Clegg *et al.*, 1978a) and an equally "compelling case" for net heterozygote disadvantage can be made. Under this hypothesis, one can assume a founding population in panmictic equilibrium. Subsequent selection steadily takes place for increasing microhabitat differentiation, and as the population ages, the seedlings which enjoy an advantage in germination and establishment in competition with their older conspecifics, are those increasingly homozygous. Indeed, significant association between allele frequencies at loci and edaphic variables was found (Schaal, 1975). Thus together with data on genotypic frequencies among zygotes and on mating which Clegg *et al.* (1978a) suggest, what is also needed is another census to establish the genotypic specificity mortality schedules of the aging cohorts. Nevertheless, this study amply demonstrates the power and potential of genetic demography in studying microevolution.

Conclusions

While lagging behind the sheer volume of studies of genetic variation in animal populations (Nevo, 1978), it is clear that a decade of research on enzyme polymorphism in plant populations has yielded a substantial and varied body of literature. The plant studies have mainly been orientated towards particular evolutionary or ecogenetic questions. They have in part exploited the diversity of breeding systems evident in the plant kingdom.

Here, Table I summarized the data concerning the genotypic structure of local populations with particular emphasis on the observed and anticipated levels of individual heterozygosity. From the overall empirical picture, there emerged the *heterozygosity paradox*—namely that in outbreeding species, heterozygosity levels are high, yet not as high as expected under the Hardy-Weinberg Law, for the level of polymorphism found. Inbreeding species, on the contrary, frequently exhibit levels of heterozygosity in excess of that expected from inbreeding theory at equilibrium in the absence of selection. This result is paradoxical because the trends run counter to those anticipated from the evolutionary pressures on mating systems. High levels of outbreeding should be favored when heterozygosity enjoys an advantage. Genes promoting selfing should increase when there is no advantage to heterozygosity and hence no inbreeding depression.

From a study of the several theoretical mechanisms that affect genotypic frequencies, three important forces may provide the basis for a solution to this paradox. These three forces are "some of the evolutionary consequences of being a plant" (Bradshaw, 1972). First, plant populations possess a remarkable capacity to fracture into very small units on a microgeographic scale, and to differentiate adaptively with respect to variation in microenvironmental selective pressures. Hence the Wahlund effect is always likely to be present. Second, partial inbreeding due to restricted neighborhood size from limited gene flow, and to self compatibility is of importance even in predominantly outbreeding species. These two forces decrease heterozygosity. Third, increasing levels of self fertilization impart an increasing coherence to the genome so that overdominance in fitness for linked segments is increasingly reinforced. The evidence reviewed here of the much greater degree of multilocus associations in inbreeding populations compared with outbreeding species is a significant accompaniment of this third force. The importance of historical or colonizing events in initiating such associations should not be neglected.

Inbreeders frequently show less variation per population than outbreeders (Table 5). However that which inbreeders do show is more markedly differentiated on a geographic (Table 5) or microgeographic scale (Table 4). Therefore one might expect that the first two forces which decrease heterozygosity, would be more marked in inbreeders than in outbreeders in lowering heterozygosity. Apparently, from Eq. (4), their scope is more limited

in inbreeders, and the third selective force is sufficient to override this effect.

If progress is to be made beyond a study of F statistics, it is from studies of selection components and genetic demography that such selective forces can be given a more physiological and ecological context. A promising start has been made in this field with a variety of phenomena already documented (Section E above). Yet, it is already clear from this review that mating systems indeed hold a key place in the study of plant populations as Lewontin noted. In this connection four points need stressing. First, reliable estimates of mating system parameters are a fundamental prerequisite of plant studies. Second, we need to go beyond the simple dichotomy of inbreeders Vs. outbreeders by making allowance for the complexity of the diverse mating systems of different plant species. There has been an undue reliance on simple experimental models (see Harding (1975) for an exception). Third, mating systems are flexible in space and in time, and such variation is likely to have profound effects on the genetic structure of populations. Greater attention should be paid to this phenomenon. Fourth, variation in mating systems is subject to genetic control, and mating systems themselves evolve. This aspect needs to be further incorporated into dynamical evolutionary models.

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