Is the Gene the Unit of Selection? Evidence from Two Experimental Plant Populations

(barley/enzyme polymorphisms/gametic phase disequilibrium/linkage disequilibrium/
natural selection/coadaptation)

M. T. CLEGG*, R. W. ALLARD, AND A. L. KAHLER

Department of Genetics, University of California, Davis, Calif. 95616

ABSTRACT   The dynamics of gametic frequency change have been analyzed in two experimental plant populations. Individual plants were scored for their genotype at four enzyme loci, and four-locus joint gametic frequencies were estimated from the genotypic data over generations. Striking correlations developed in allelic state over loci, including correlations between nonlinked loci, as these populations evolved. Furthermore, the same pair of four-locus complementary gametic types came into marked excess in the late generations of both populations. The results demonstrate that natural selection acted to structure the genetic resources of these populations into sets of highly interacting, coadapted gene complexes. They also provide evidence, at the level of the gene, that selection operating on correlated multilocus units is an important determinant of population structure.

Sexual reproduction and genetic recombination are capable of producing a virtually infinite array of genotypes. The potential diversity is especially striking in view of current estimates that indicate that at least one-third of electrophoretically detectable loci are polymorphic (1–4). A particularly challenging issue in population genetics is to determine how natural selection sorts out from the vast number of potential arrays of genotypes those arrays that confer high fitness on the population. Measurement of correlations in allelic state over loci in the gametic pool provides an important test of the extent to which natural selection reduces the potential genotypic frequency distribution.

Theoretical and numerical investigations show that complex relationships exist between selection, linkage, effective population size, and mating system when two or more loci are considered simultaneously. Exact treatments of two-locus symmetric viability models in which random mating and infinite population size are assumed show that up to seven interior equilibria are possible, and that equilibrium points are given by complex functions of the recombination fraction and viabilities (5). If population size is finite, recombination is restricted and, through the vagaries of sampling, wide fluctuations in disequilibria may result (6). Inbreeding due to mating system also reduces effective recombination by lowering levels of heterozygosity, and very close inbreeding leads to drastic restriction of recombination with effects on equilibria equivalent to those of very tight linkage (7).

Numerical investigations suggest that additional phenomena become involved when more than two, and especially when large numbers of, loci segregate simultaneously. Thus, Sved (6) finds that apparent single-locus selective values are much greater than actual selective values due to reinforcement of selection intensity over loci along the chromosome. Franklin and Lewontin (8), assuming multiplicative heterosis, find that when recombination falls within certain critical ranges high correlations in allelic state accumulate rapidly along the chromosome; the effect of these correlations is to restrict the gametic pool to few gametic types, usually perfectly complementary to one another. These authors speculate that selection acts, not on single loci, but upon chromosomal blocks composed of highly interacting sets of loci.

Theoretical analyses of models involving two or more loci thus make it clear that the behavior of multilocus systems cannot, in general, be predicted from single-locus theory. There is, however, a paucity of experimental data on the dynamics of multilocus systems, especially for systems involving more than two loci. Predominantly self-pollinating populations of plants are favorable materials for such studies, since the intense inbreeding that occurs in such populations is expected to lead to drastic restriction of recombination among all loci, including unlinked loci, with effects on equilibria equivalent to very tight linkage. This paper reports a study of gametic frequency change at four enzyme loci in two experimental populations of barley (*Hordeum vulgare* L.), in which the mating system is one of more than 99% self-fertilization. The results show clearly the buildup of striking correlations between loci, including unlinked loci, and they provide experimental evidence, at the level of the gene, that selection operating on such coadapted (9) multilocus units is an important determinant of population structure.

MATERIALS AND METHODS

The two experimental plant populations studied are barley Composite Crosses II and V (hereafter abbreviated CCII and CCV). CCV was synthesized from 30 parents representing the major barley growing regions of the world (10). The parents were crossed in all possible pairs, the resulting F_1 hybrids were again paircrossed, and this cycle was repeated until a single grand F_1 hybrid was obtained. The F_2 seed from the grand F_1 hybrid was used to initiate the first generation of CCV in 1941. The population has since been propagated in large plots every year under normal agricultural conditions without conscious selection. Examination of the gametic input of the parental varieties indicates that frequencies of the various gametic types in the initial generation of this population deviated in a minor way from products of single-locus allelic frequencies. This initial gametic phase disequilibrium (also called linkage disequilibrium, which is a misnomer because disequilibrium can occur without linkage) was of about the magnitude expected from sampling effects associated with the small number of parents (11).

CCII differs from CCV both in parentage and in method of synthesis. The population was initiated in 1929 by pooling equal numbers of F_1 hybrid seed from the 378 intercrosses among its 28 parents, which, like the parents of CCV, included a wide sample of the diversity of cultivated barley. Population size and cultural practices were the same for this population as for CCV. Analyses of the gametic input of the parental varieties indicates slightly greater gametic phase disequilibrium in the initial generation of CCII than in CCV. Moreover, there was less opportunity for recombination during the synthesis of CCII than during the synthesis of CCV; thus, the initial generation of CCII almost certainly deviated further from gametic phase equilibrium than the initial generation of CCV.

Four esterase loci, EA, EB, EC, and ED (hereafter designated A, B, C, D) were simultaneously scored in each individual assayed in generations 5–7, 16–17, and 25–26 in CCV and 7–10, 17–20, and 39–42 in CCII. The formal genetics and assay techniques have been described (12). Three of these loci are very tightly linked, in the order B←0.0023 ± 0.0007 →A←0.0048 ± 0.0008→C, while the fourth locus, D, is inherited independently of the first three.

Census data were obtained by analysis of 7-day-old seedlings grown from the bulk seed harvest of the generations selected for study. The seeds were germinated under ideal conditions and mortality was very low (<1%). Hence, the census data obtained are effectively equivalent to enumerations made at the zygotic stage.

RESULTS

The number of alleles of Loci A, B, C, and D introduced into CCII and CCV (10) were 3, 5, 3, and 5, and 4, 3, 3, and 4, respectively. In both populations one allele generally predominated at each locus and one or more of the other alleles was present in low frequency (the predominant allele was the same at each locus in the initial generation of both populations). With such substantial allelic diversity, the number of four-locus joint genotypes is very large. Consequently, to

TABLE 1. *Relative gametic frequencies and their relative deviations (in parentheses) from products of allelic frequencies for one early, one intermediate, and one late generation of CCV*

Gamete	Generation		
	5	17	26
1111	0.125 (−0.012)	0.173 (+0.004)	0.126 (−0.027)
1112	0.185 (+0.009)	0.122 (−0.025)	0.052 (−0.005)
1121	0.078 (+0.004)	0.081 (−0.025)	0.110 (−0.066)
1122	0.078 (−0.016)	0.119 (+0.027)	0.067 (+0.001)
1211	0.003 (−0.009)	0.000 (−0.016)	0.005 (−0.049)
1212	0.004 (−0.011)	0.000 (−0.014)	0.000 (−0.020)
1221	0.025 (+0.019)	0.054 (+0.044)	0.229 (+0.167)
1222	0.025 (+0.016)	0.013 (+0.004)	0.022 (−0.001)
2111	0.131 (+0.006)	0.171 (+0.040)	0.175 (+0.078)
2112	0.180 (+0.019)	0.129 (+0.015)	0.106 (+0.070)
2121	0.071 (+0.004)	0.052 (−0.030)	0.081 (−0.031)
2122	0.072 (−0.014)	0.065 (−0.006)	0.022 (−0.019)
2211	0.005 (−0.006)	0.004 (−0.008)	0.000 (−0.034)
2212	0.017 (+0.004)	0.015 (+0.004)	0.001 (−0.012)
2221	0.000 (−0.006)	0.000 (−0.007)	0.002 (−0.038)
2222	0.000 (−0.007)	0.000 (−0.007)	0.001 (−0.014)
N^*	1452	2443	3049

*N denotes the number of zygotes observed.

reduce the data to more managable proportions, we have combined alleles to create two allelic classes per locus, one consisting of the most-frequent allele (denoted allele 1) and the other a "synthetic" allele consisting of all other alleles combined (denoted allele 2).

Calculation of gametic input

In calculating gametic inputs, it is convenient to adopt and extend the notation of Weir *et al.* (11). The relative frequency of a heterozygous genotype is written as $2f(A_iB_jC_kD_1,A_nB_m-C_pD_q)$, where genes $A_iB_jC_kD_1$ were contributed by the same parental gamete, and the relative frequency of a four-locus joint homozygote is denoted $f((A_iB_jC_kD_1,A_iB_jC_kD_1)$. Thus, the relative frequency of a particular four-locus gamete can be found from the sums of appropriate genotypic frequencies. For example in the diallelic case,

$$f(A_1B_1C_1D_1) = f(A_1B_1C_1D_1,A_1B_1C_1D_1) + f(A_1B_1C_1D_1,A_1B_1C_1D_2) +$$
$$f(A_1B_1C_1D_1,A_1B_1C_2D_1) + f(A_1B_1C_1D_1,A_1B_2C_1D_1) +$$
$$f(A_1B_1C_1D_1,A_2B_1C_1D_1) + f(A_1B_1C_1D_1,A_1B_1C_2D_2) +$$
$$f(A_1B_1C_1D_1,A_1B_2C_1D_2) + f(A_1B_1C_1D_1,A_2B_1C_1D_2) +$$
$$f(A_1B_1C_1D_1,A_1B_2C_2D_1) + f(A_1B_1C_1D_1,A_2B_1C_2D_1) +$$
$$f(A_1B_1C_1D_1,A_2B_2C_1D_1) + f(A_1B_1C_1D_1,A_1B_2C_2D_2) +$$
$$f(A_1B_1C_1D_1,A_2B_1C_2D_2) + f(A_1B_1C_1D_1,A_2B_2C_1D_2) +$$
$$f(A_1B_1C_1D_1,A_2B_2C_2D_1) + f(A_1B_1C_1D_1,A_2B_2C_2D_2),$$

or, in general, $f(ijkl) = \sum_n \sum_m \sum_p \sum_q f(ijkl,nmpq)$.

Since progeny testing to determine the frequencies of the various phases of multiply heterozygous individuals was impractical, the frequencies of such genotypes are unknown; consequently, in computing gametic input, we have assumed that the various phases of multiple heterozygotes were present in equal frequency. For example, in the case of the triple heterozygote it is assumed that $f(A_iB_jC_kD_1,A_iB_nC_mD_p) = f(A_iB_nC_kD_1,A_iB_jC_mD_p) = f(A_iB_jC_mD_1,A_iB_nC_kD_p) = f(A_i-B_jC_kD_p,A_iB_nC_mD_1) = \frac{1}{8}f(A_iA_iB_jB_nC_kC_mD_1D_p)$. Weir *et al.* (11) have shown that errors resulting from the assumption of phase equality are negligible because the heavy self-fertilization in these populations (>99%) causes the frequency of multiple heterozygotes to be very low. However, errors due to this assumption will lead to underestimates of the frequencies of gametic types that are in excess.

Since results are similar within the early, intermediate, and late generations of both populations, data will be given for only a single generation in each group. Table 1 displays the sixteen four-locus relative gametic frequencies and the relative deviations of each gametic frequency from the product of allelic frequencies in generations 5, 17, and 26 of CCV. The numbers of individuals assayed in each generation are large, so that the frequencies of the 16 gametic types have been estimated very accurately. There are eight negative and eight positive deviations in generation 5; however, by generation 26 twelve of the 16 deviations are negative and only three of the gametes are present in large excess. These three favored gametes account for more than 50% of the gametes in the gametic pool; furthermore, they are perfectly complementary for the A-B-C loci. Two of the favored gametic types, $A^{(1)}B^{(2)}-C^{(2)}D^{(1)}$ (abbreviated 1221) and $A^{(2)}B^{(1)}C^{(1)}D^{(2)}$ (2112), which are perfectly complementary over all four loci, account for more than 30% of the gametic pool.

To measure the extent to which interactions accumulate between loci in these populations, chi-square statistics were

TABLE 2. *Chi-square values calculated as deviations from products of allelic frequencies for all two-locus gametes, three-locus gametes, and four-locus gametes in CCV*

	Generation		
	5	17	26
Locus pair (χ^2_1)			
A-B	18.4	43.6	633.6
A-C	13.4	107.6	534.9
B-C	44.0	150.2	837.6
A-D	0.1	1.7	38.9
B-D	0.2	17.4	171.2
C-D	11.4	14.9	66.2
Locus triplet (χ^2_4)			
A-B-C	170.4	463.9	2091.7
A-B-D	23.6	117.6	894.3
A-C-D	25.0	124.2	649.6
B-C-D	57.3	302.8	1126.6
Locus quadruplet (χ^2_{11})			
A-B-C-D	189.0	699.4	2601.7

$P(\chi^2_1 \geq 3.84) \leq 0.05; P(\chi^2_4 \geq 9.49) \leq 0.05; P(\chi^2_{11} \geq 19.68) \leq 0.05.$

computed from the gametic contingency tables for all two-locus, three-locus, and four-locus gametes. Table 2 presents the chi-square statistics for CCV, from which it can be seen that highly significant departures from random association of alleles at the different loci have developed for the tightly linked loci by generation 5. Even more striking, however, is the dramatic increase in departure from randomness over time. By generation 26 all loci interact very strongly, including the unlinked D locus. This kind of behavior is not compatible with the expected dynamics of neutral loci.

Gametic frequencies for CCII, given in Table 3, show that the behavior of this population parallels that of CCV in several important respects. Perhaps of greatest significance is

TABLE 3. *Relative gametic frequencies and their relative deviations (in parentheses) from products of allelic frequencies for one early, one intermediate, and one late generation in CCII*

	Generation		
Gamete	7	18	41
1111	0.096 (−0.056)	0.058 (−0.070)	0.004 (−0.018)
1112	0.084 (−0.017)	0.150 (+0.077)	0.061 (−0.034)
1121	0.097 (−0.004)	0.184 (+0.008)	0.014 (+0.011)
1122	0.074 (+0.007)	0.032 (−0.070)	0.002 (−0.013)
1211	0.002 (−0.022)	0.000 (−0.017)	0.000 (−0.001)
1212	0.000 (−0.016)	0.000 (−0.010)	0.000 (−0.006)
1221	0.129 (+0.112)	0.107 (+0.082)	0.060 (+0.060)
1222	0.007 (−0.004)	0.014 (−0.000)	0.003 (+0.002)
2111	0.193 (+0.035)	0.075 (−0.032)	0.054 (−0.074)
2112	0.224 (+0.119)	0.136 (+0.075)	0.746 (+0.181)
2121	0.081 (−0.024)	0.210 (+0.064)	0.052 (+0.032)
2122	0.010 (−0.060)	0.032 (−0.052)	0.004 (−0.084)
2211	0.001 (−0.024)	0.000 (−0.015)	0.000 (−0.008)
2212	0.000 (−0.017)	0.000 (−0.008)	0.000 (−0.038)
2221	0.000 (−0.017)	0.000 (−0.020)	0.000 (−0.001)
2222	0.000 (−0.011)	0.000 (−0.012)	0.000 (−0.006)
N^*	1044	2087	2868

$^*N$ denotes the number of zygotes observed.

that the 1221 and 2112 complementary gametic types, which are favored in CCV, are also favored in CCII. The trajectory of gametic frequency change is more complicated in CCII, in that the 2112 gamete decreased in frequency in the intermediate generations before assuming very high frequency in the latest generations. Chi-square values for CCII (Table 4) show that departures from randomness were larger in the early generations of this population than in CCV. Although little further nonrandomness developed between the early and middle generations in CCII, very large increases occurred between middle and late generations. However, the latter observation must be interpreted cautiously, since the four-locus gametic chi-square in the latest generations may be biased by the low expected number of one of the gametic types (1221).

To obtain an idea of the effect of consolidating the data into two allelic classes, we have also estimated the frequency of all 81 triallelic four-locus gametic types. In the triallelic classification, the two initially most frequent alleles in CCV were designated alleles 1 and 2, and the remaining rare alleles were combined into a new synthetic allele, designated allele 3. Table 5 presents the frequencies of those triallelic gametes that were most favored in the late generations of CCV and CCII. Among the 81 possible triallelic gametes, two of the three most favored ones (1221 and 2112) are identical in both populations. Since these are the same gametic types that were the most favored among the diallelic gametes, the consolidation of alleles has not distorted the picture of the dynamics of gametic frequency change.

The complete triallelic data do, however, yield additional information. In generation 5 of CCV, 24 of the 81 gametes show positive deviations and 17 gametes still show positive deviations in generation 26. Evidently, more nonrandomness had accumulated than was revealed by the diallelic analysis. In both populations, gametes with allele 3 at the A locus are usually deficient, suggesting that the rarer alleles at this locus combine poorly with all allelic combinations at the other loci.

Chi-square statistics could not be computed for the tri-

TABLE 4. *Chi-square values calculated as deviations from products of allelic frequencies for all two-locus gametes, three-locus gametes, and four-locus gametes in CCII*

	Generation		
	7	18	41
Locus pair (χ^2_1)			
A-B	167.8	238.1	1150.2
A-C	217.7	14.4	711.6
B-C	235.2	204.3	1229.4
A-D	15.9	0.3	415.1
B-D	87.2	77.3	761.8
C-D	84.2	657.1	1672.4
Locus triplet (χ^2_4)			
A-B-C	716.4	591.4	9245.9
A-B-D	329.7	376.0	6138.9
A-C-D	319.0	672.8	4723.5
B-C-D	459.3	943.0	7100.7
Locus quadruplet (χ^2_{11})			
A-B-C-D	1152.3	1432.6	46,046.6

$P(\chi^2_1 \geq 3.84) \leq 0.05; P(\chi^2_4 \geq 9.94) \leq 0.05; P(\chi^2_{11} \geq 19.68) \leq 0.05.$

allelic gametes, because expected numbers were too low in many classes. Hence, to quantify the triallelic disequilibria we have used a parameter of gametic phase disequilibrium, defined as $d_{ij} = g_{ij} - p_iq_j$ (13), where g_{ij} is the relative frequency of a two-locus gamete with the ith allele at one locus and the jth allele at the second locus; p_i and q_j denote the relative frequency of the ith and jth alleles at the first and second loci, respectively. Since d_{ij} depends on gene frequencies, we express it relative to the most extreme value it can take for a given gene frequency:

$$d'_{ij} = \frac{d_{ij}}{p_iq_j} \text{ if } d_{ij} < 0$$

or

$$d'_{ij} = \frac{d_{ij}}{p_i(1 - q_j)} \text{ or } \frac{d_{ij}}{q_j(1 - p_i)},$$

whichever is greater, if $d_{ij} > 0$. The total relative gametic phase disequilibrium for any two-locus pair, say A-B, is measured as

$$D'_{AB} = \sum_{ij} |d'^{(AB)}_{ij}| g^{(AB)}_{ij},$$

a weighted mean over gametic types. Table 6 displays the D′ estimates for all six two-locus pairs in both populations. In CCII D̄′ reached over 50% of its maximal value by generation 7, after which there was a slight decline through the intermediate generations, followed by an increase to more than 75% of maximum possible disequilibrium in generation 41. In CCV regular increases occurred in D̄′ from the early to the late generations, by which time the population had achieved nearly 50% of maximum disequilibrium. In both populations the very tightly linked B-A-C loci show generally larger interactions with each other than with the unlinked D locus. Among the three tightly linked loci, the A locus shows the smallest pairwise interactions in its combinations with the other three loci. Since the A locus lies between loci B and C on the linkage map, the differential tendency of the three loci to form correlated pairs suggests that it is the effects of interactions among these specific loci that are being measured.

TABLE 5. *Most favored triallelic four-locus gametic types and their relative deviation from products of allelic frequencies in CCII and CCV*

Gamete	CC II Generation		
	7	18	41
1221	0.071 (+0.067)	0.062 (+0.055)	0.052 (+0.051)
2112	0.109 (+0.068)	0.021 (+0.013)	0.497 (+0.119)
2113	0.115 (+0.054)	0.115 (+0.070)	0.248 (+0.062)
N	1044	2087	2867

Gamete	CC V Generation		
	5	17	26
1221	0.009 (+0.009)	0.030 (+0.029)	0.173 (+0.151)
2111	0.129 (+0.034)	0.170 (+0.068)	0.173 (+0.092)
2112	0.038 (+0.006)	0.063 (+0.029)	0.085 (+0.066)
N	1452	2443	3049

TABLE 6. *Parameter of gametic phase disequilibrium, D′, and its mean, D̄′, over locus pairs in CCII and CCV*

Locus pair	CC II Generation		
	7	18	41
A-B	0.690	0.622	0.946
A-C	0.548	0.324	0.520
B-C	0.821	0.839	0.966
A-D	0.141	0.108	0.424
B-D	0.513	0.351	0.856
C-D	0.383	0.607	0.900
D̄′	0.516	0.475	0.769

Locus pair	CC V Generation		
	5	17	26
A-B	0.235	0.264	0.698
A-C	0.420	0.503	0.574
B-C	0.324	0.643	0.856
A-D	0.047	0.071	0.153
B-D	0.090	0.159	0.378
C-D	0.105	0.162	0.286
D̄′	0.204	0.300	0.491

DISCUSSION

Two aspects of gametic frequency change in CCII and CCV make it clear that natural selection was the primary force responsible for the organization of the genetic materials of these two populations into highly correlated units. [It has been shown by Allard *et al.* (10) that mutation, migration, and genetic drift can have had no more than inconsequential effects on genetic change over generations.] First, departures from random association of alleles within gametes increased over generations in both populations. These increases cannot be reconciled with neutrality, because any initial nonrandomness among neutral units is expected to decrease over generations (14), rather than increase as occurred in CCII and CCV. Such increases in nonrandomness are, however, compatible with selection favoring particular combinations of alleles. Second, the same two balanced four-locus gametes come into greatest excess in both populations. The probability that the same two gametes (among 225 and 144 possible gametic types in CCII and CCV, respectively) should come into excess in two separate populations, synthesized from different parents by different methods, is very small unless these two gametic types represent mutually concordant gene complexes strongly favored by selection.

Although the changes that occurred in CCII and CCV provide strong evidence that selection altered associations among alleles of the four enzyme loci, these changes do not in themselves establish whether the enzyme loci are the unit of selection or whether they are neutral markers for blocks of genes surrounding each locus. However, the botanical history of the A, B, C, and D locus polymorphisms provide evidence on this point. The alleles at these four loci that were monitored in the two populations are found in the wild progenitor of barley, and these alleles also have a world-wide distribution in cultivated barley (Kahler and Allard, unpublished data). Thus, the polymorphisms in question are evidently ancient ones, and in such long-established polymorphisms it is ex-

pected that *neutral* isoalleles will be in equilibrium with alleles at all other loci. Thus, if the A, B, C, and D locus alleles are neutral, it is expected that they will be randomly distributed with respect to background genotype in the barley species as a whole. It is also expected that vagaries of sampling associated with the synthesis of CCII and CCV from small samples of the total variability of the barley species (28 and 30 parents, respectively, for CCII and CCV) might cause particular A, B, C, and D locus alleles to be associated nonrandomly with respect to blocks of genes that are favored in the environment of Davis, California. This in turn might have led to correlations among the alleles of the enzyme loci as selection organized the associated loci into interacting complexes. However, it is highly unlikely that the same sampling accident would occur in both populations. Thus, the fact that the same four-locus combination of allozymes was favored in both populations indicates that the enzyme loci are not mere neutral markers but that they themselves contribute to the aggregate effect on fitness of the block of genes in which they are embedded.

The development and maintenance of such highly correlated gene complexes in these two populations also highlight the critical relationship between selection and restriction of recombination in the structuring of genetic materials. Favorable combinations of alleles involving the A, B, and C loci are protected from breakup by the very tight linkages between these three loci. However, favorable associations involving the unlinked D locus are not so protected and the fact that this locus was incorporated into the four-locus complex shows that restriction of recombination due to close inbreeding can also prevent breakup of favorable associations of alleles. Since inbreeding restricts recombination between all loci, whether on the same or different chromosomes, it presumably ties the entire genotype together. Surveys of additional loci in these two populations should throw light on the interesting question of whether correlations exist between all loci in CCII and CCV, and in all heavily inbreeding populations.

It is now evident that a high proportion of populations of predominantly self-fertilizing plants maintain very large stores of genetic variability, not only for enzyme loci (4, 10) but also for genes governing morphological polymorphisms and quantitative characters (15). The maintenance of such substantial genetic variation has been most difficult to explain within the framework of single-locus theory. In barley, for example, which is more than 99% self-fertilized (10), the reproductive value of heterozygotes must be nearly double the mean of the reproductive values of the homozygotes for stable nontrivial polymorphisms to exist (16). Thus, the number of polymorphisms that can be maintained by single-locus heterozygote advantage is clearly very limited in heavily inbreeding populations. The persistence of extensive variability in such populations is, however, compatible with the occurrence of marked excesses of perfectly complementary multilocus gametic types of the kind found in the present study. So also are the moderate excesses of heterozygotes that have been found in CCV (10) and in other inbreeding populations (15, 17).

Although the present study does not establish the mode of selection, previous studies indicate that several types of selection may be involved in the development and maintenance of coadapted blocks of genes. Examination of two-locus zygotic arrays in CCV showed that each allele at any one locus is favored in at least one combination, and at a disadvantage in at least one combination with alleles at each other locus (11). Such epistatic interactions lead to frequency-dependent selection of a type that might favor the development of balanced gene complexes. Another study of CCV revealed that reproductive capacity is often higher when individuals compete with genotypes other than their own (18). Such intergenotypic interactions also lead to a frequency-dependent type of selection that could promote the development of coadapted blocks of genes. Still another type of selection was indicated in a study of natural populations of the slender wild oat, *Avena barbata;* in this study, multiniche selection appeared to contribute to marked excesses of complementary five-locus gametic types similar to those observed in CCII and CCV. Two main features emerge from the present study: (*i*) that selection, whatever its form or forms, acts to structure the genetic materials into highly interacting sets of loci, or coadapted gene complexes (9); (*ii*) that this has the effect of reducing the enormously large potential genotypic frequency distribution in ways such that those genotypes that confer high fitness occur much more frequently than would be expected if the genes act independently of one another.

1. Ayala, F. J., Powell, J. R. & Dobzhansky, Th. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2480–2483.
2. Selander, R. K., Hunt, W. G. & Yang, S. Y. (1969) *Evolution* **23**, 379–390.
3. Harris, H. (1966) *Proc. Roy. Soc. Ser. B* **164**, 298–310.
4. Allard, R. W. & Kahler, A. L. (1971) in *Stadler Symposia* (Univ. of Missouri), Vol. 3, pp. 9–24.
5. Karlin, S. & Feldman, M. W. (1970) *Theor. Pop. Biol.* **1**, 39–71.
6. Sved, J. A. (1968) *Genetics* **59**, 543–563.
7. Jain, S. K. & Allard, R. W. (1966) *Genetics* **53**, 633–659.
8. Franklin, I. & Lewontin, R. C. (1970) *Genetics* **65**, 707–734.
9. Dobzhansky, Th. (1970) *Genetics of the Evolutionary Process* (Columbia University Press, New York).
10. Allard, R. W., Kahler, A. L. & Weir, B. S., *Genetics*, in press.
11. Weir, B. S., Allard, R. W. & Kahler, A. L., *Genetics*, in press.
12. Kahler, A. L. & Allard, R. W. (1970) *Crop Sci.* **10**, 444–448.
13. Brown, A. H. D. (1969) *The effect of reciprocal recurrent selection for yield on isozyme polymorphisms in maize*, Ph.D. Thesis, University of California, Davis.
14. Karlin, S. (1969) in *Equilibrium Behavior of Population Genetic Models with Non-random Mating* (Gordon and Breach, Science Publishers, New York), pp. 21–23.
15. Allard, R. W., Jain, S. K. & Workman, P. L. (1968) *Advan.*
16. Hayman, B. I. (1953) *Heredity* **7**, 185–192.
17. Marshall, D. R. & Allard, R. W. (1970) *Genetics* **66**, 393–399.
18. Allard, R. W. & Adams, J. (1969) *Amer. Natur.* **103**, 621–645.