Gametic Disequilibrium Measures: Proceed With Caution

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ABSTRACT

Five different measures of gametic disequilibrium in current use and a new one based on R. C. Lewontin's D', are examined and compared. All of them, except the measure based on Lewontin's D', are highly dependent upon allelic frequencies, including four measures that are normalized in some manner. In addition, the measures suggested by A. H. D. Brown, M. F. Feldman and E. Nevo, and T. Ohta can have negative values when there is maximum disequilibrium and have rates of decay in infinite populations that are a function of the initial gametic array. The variances were large for all the measures in samples taken from populations at equilibrium under neutrality, with the measure based on D' having the lowest variance. In these samples, three of the measures were highly correlated, D^2, D^* (equal to the correlation coefficient when there are two alleles at each locus) and the measure $X(2)$ of Brown *et al.* Using frequency-dependent measures may result in mistaken conclusions, a fact illustrated by discussion of studies inferring recombinational hot spots and the effects of population bottlenecks from disequilibrium values.

WITH the advent of new biochemical techniques, it has been possible to distinguish many alleles at a number of loci and to document DNA differences at closely linked sites on a chromosome. If population data from such studies are examined, the extent of the statistical association of the alleles at different loci or sites, gametic disequilibrium, may be an indicator of the past importance of various evolutionary factors. However, these are a variety of gametic disequilibrium measures that have been proposed by different researchers.

There are a number of criteria that can be used to determine the most appropriate measure of gametic disequilibrium for a given situation. Several possible criteria are that a measure should have (1) a simple biological interpretation, (2) statistical tests available or easily developed, (3) be directly related mathematically to evolutionary factors such as recombination, selection, genetic drift, gene flow, etc. and (4) be standardized to allow comparison across loci or populations. Obviously, all of these criteria (and probably more) are important characteristics for a disequilibrium measure. In addition, it is not obvious whether different measures gave similar information, or whether different measures may give complementary information.

Below I will examine six different measures of two-locus gametic disequilibrium. First, I have compared some of the basic properties of the different measures focusing on the allelic frequency dependence of the measures and the rate of decay of disequilibrium from recombination. Second, I have compared the distributions of these measures and calculated the correlation coefficients between them for a large number of randomly obtained samples.

MEASURES OF GAMETIC DISEQUILIBRIUM

The extent of gametic disequilibrium (I use the term gametic disequilibrium instead of the traditional term linkage disequilibrium because such nonrandom association may be present between unlinked loci) [see HEDRICK, JAIN and HOLDEN (1978) for a discussion] can be measured in several ways for a specific gamete. A widely used measure of gametic disequilibrium for a given gamete is

$$D_{ij} = x_{ij} - p_i q_j \tag{1}$$

where x_{ij} is the observed frequency of gamete A_iB_j, p_i and q_j are the frequencies of alleles A_i and B_j at loci A and B, respectively, and the expected frequency of gamete A_iB_j is p_iq_j, assuming no statistical association between the alleles. The range of this measure of gametic disequilibrium is a function of the allelic frequencies, making it obvious that a measure that has the same range for all allelic frequencies is desirable. For this reason, LEWONTIN (1964) suggested using the normalized measure

$$D'_{ij} = \frac{D_{ij}}{D_{\max}} \tag{2}$$

where $D_{\max} = \min[p_iq_j, (1 - p_i)(1 - q_j)]$ when $D_{ij} < 0$ or $D_{\max} = \min[p_i(1 - q_j), (1 - p_i)q_j]$ when $D_{ij} > 0$.

The gametic disequilibrium between all the alleles at two loci can be measured in several ways. For

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example, the disequilibrium can be expressed as

$$D^2 = \sum_{i=1}^{k} \sum_{j=1}^{l} D_{ij}^2 \tag{3}$$

where there are k alleles at locus A and l alleles at locus B. However, this measure, like D_{ij}, is highly dependent upon allelic frequencies. As a result, other measures of association in which the overall disequilibrium is normalized and not so dependent on allelic frequencies have been suggested.

One such approach is to standardize the measure by the single-locus heterozygosity. For example, the Hardy-Weinberg homozygosity at locus A having k alleles is

$$F_A = \sum_{i=1}^{k} p_i^2 \tag{4a}$$

and for locus B with l alleles is

$$F_B = \sum_{j=1}^{l} q_j^2. \tag{4b}$$

The Hardy-Weinberg heterozygosities, $H_A = 1 - F_A$ and $H_B = 1 - F_B$, can be used in a standardized measure of two-locus association as

$$D^* = \frac{D^2}{H_A H_B} \tag{5a}$$

a measure termed R by MARUYAMA (1982).

First, note that when there are two alleles at both loci that

$$D^* = \frac{D^2}{4p_1(1 - p_1)q_1(1 - q_1)}. \tag{5b}$$

Because all D_{ij} are equal with two alleles at both loci, then $D^2 = 4D_{ij}^2$ and D^* is equal to the square of the correlation coefficient as used by HILL and ROBERTSON (1968) and FRANKLIN and LEWONTIN (1970). Second, note that expression (5a) is different from σ_D^2 as given by HILL (1975) and OHTA (1980). D^*, when calculated over a number of samples, is the mean of the ratio in (5a) whereas σ_D^2 is the ratio of the means (or expectations), an important difference in many situations (MARUYAMA 1982; HEDRICK and THOMSON 1986, Table 4). Obviously, σ_D^2 is mathematically more tractable but D^* is the experimentally observable quantity in each population or pair of loci (MARUYAMA 1982).

Another measure of standardized gametic disequilibrium is

$$Q = n \sum_{i=1}^{k} \sum_{j=1}^{l} \frac{D_{ij}^2}{p_i q_j} \tag{6}$$

(HILL 1975). In order to make this measure sample-size independent when $D \neq 0$ (BISHOP, FEINBERG and HOLLAND 1975), the measure

$$Q^* = \frac{1}{(k - 1)(l - 1)} \sum_{i=1}^{k} \sum_{j=1}^{l} \frac{D_{ij}^2}{p_i q_j} \tag{7}$$

is useful (HEDRICK and THOMSON 1986). D^* and Q^* are equal for $k = l = 2$. YAMAZAKI (1977) proposed the related measure

$$L = \frac{n}{k - 1} \sum_{i=1}^{k} \sum_{j=1}^{l} \frac{D_{ij}^2}{p_i q_i} \tag{8}$$

where it is assumed that $k < l$. Because L is a linear function of Q^*, we will not give values for it in the following discussion.

If we define

$$F_{AB} = \sum_{i=1}^{k} \sum_{j=1}^{l} x_{ij}^2 \tag{9}$$

another measure of association is

$$F^* = F_{AB} - F_A F_B \tag{10}$$

by OHTA (1980) and is equivalent to the covariance of heterozygosity measure of AVERY and HILL (1979). OHTA (1980) suggested F^* could be standardized with the product of the Hardy-Weinberg heterozygosities as

$$F' = \frac{F^*}{H_A H_B}. \tag{11}$$

This measure is somewhat different from the standardized measure of OHTA (1980) in that for a number of samples, she uses the mean of F_{AB}, F_A and F_B over samples to calculate her measure (see discussion of D^* above).

BROWN, FELDMAN and NEVO (1980) suggested a measure of disequilibrium based on the variance of heterozygosity, higher variance of heterozygosity being the result of higher gametic disequilibrium. If it is assumed that K is the number of heterozygous loci, then for two loci $K = 0$ for the double homozygotes, $K = 1$ for the single heterozygotes and $K = 2$ for the double heterozygotes. The variance of K for two loci is then

$$s_K^2 = H_A + H_B - H_A^2 - H_B^2 + 4 \sum_{i=1}^{k} \sum_{j=1}^{l} p_i q_j D_{ij} + 2D^2 \tag{12}$$

where D^2 is as defined in expression (3). Expression (4) of SVED (1968) and (10) of AVERY and HILL (1979) are diallelic cases of (12) for multiple loci. BROWN, FELDMAN and NEVO (1980) suggested that expression (12) can be standardized into a statistic, which they term association intensity, as

$$X(2) = \frac{s_K^2}{H_A + H_B - H_A^2 - H_B^2} - 1 \tag{13}$$

where the 2 refers to the second central moment. When there is no disequilibrium, then $X(2) = 0$.

Finally, the normalized measure of LEWONTIN can also be used for a total disequilibrium measure. For example.

$$D' = \sum_{i=1}^{k} \sum_{j=1}^{l} p_i q_j \, |D'_{ij}| \qquad (14)$$

gives values of the absolute value of the normalized D weighted by the frequencies of the gametes when there is no disequilibrium. A similar measure was used by KARLIN and PIAZZA (1982) but they used x_{ij} values for weighting.

GENERAL PROPERTIES

Frequency-dependence: An important characteristic of a gametic disequilibrium measure is that it is independent (or nearly independent) of allelic frequencies. For example, a measure that is highly dependent on allelic frequencies would not be appropriate for comparisons between samples or loci with different allelic frequencies. To examine the dependence on allelic frequencies of these six measures, let us first assume that there are two alleles at each of the two loci and use D'_{ij} as a standard because it is independent of allelic frequencies. Assuming that there is a given D'_{ij} value, we can calculate the value of the different measures for various allelic frequencies at the two loci. For example, if $D'_{11} > 0$ and $p_1q_2 < p_2q_1$, then

$$D_{11} = D'_{11} \, p_1q_2 \qquad (15)$$

and the various standardized measures, D^*, Q^*, F', $X(2)$ and D' can then be calculated (remember $D^* = Q^*$ for two alleles). As an example of what the gametic frequencies are for given values, let $D'_{11} = 1$, $p_1 = 0.1$, and $q_2 = 0.5$, then $D_{11} = 0.05$, $x_{11} = p_1q_1 + D_{11} = 0.1$, $x_{12} = 0.0$, $x_{21} = 0.4$, and $x_{22} = 0.5$. The other disequilibrium measures are then $D^* = Q^* = 0.111$, $F' = 0.111$, $X(2) = 0.030$, and $D' = 1.0$.

Using this approach, the values of $D^* = Q^*$, F', and $X(2)$ are plotted in Figures 1, 2, and 3, respectively, for different allelic frequencies when $D'_{11} = 0.5$ ($D' = 0.5$ for all allelic frequency combinations). When $D'_{11} = -0.5$, complementary results occur and when $|D'_{11}| < 0.5$, then similar but not as extreme results occur. First, notice that all of these measures, even though they are standardized in some manner, are highly dependent upon allelic frequencies. For example, looking at $D^* = Q^*$ in Figure 1 and $X(2)$ in Figure 3, if one locus has both alleles at intermediate frequency, say $q_1 = q_2 = 0.5$, and the other locus has one allele in much higher frequency, say $p_1 = 0.05$ and $p_2 = 0.95$, then the calculated values are much lower than if the alleles at both loci were intermediate in frequency.

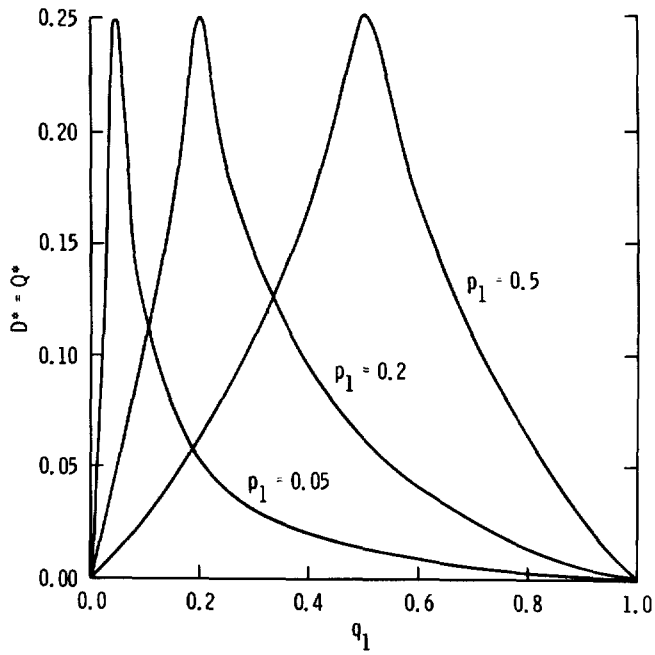

FIGURE 1.—Magnitude of D^* for different p_1 and q_1 values when $D' = 0.5$.

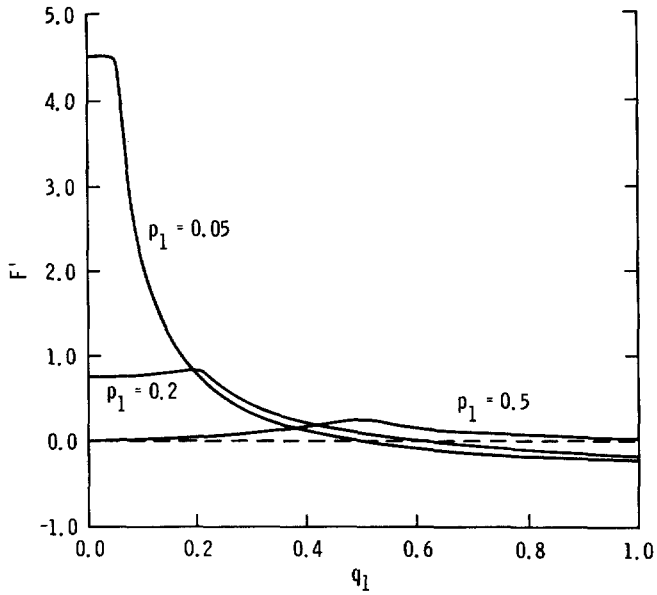

FIGURE 2.—Magnitude of F' for different p_1 and q_1 values when $D' = 0.5$.

Second, notice that the maximum values for each measure occur when the allelic frequencies at the two loci are equal. For $D^* = Q^*$, this maximum is the same for all $p_1 = q_1$ values (Figure 1). However, for F' and $X(2)$ the maximum is greatest when the allelic frequencies are low, *i.e.*, F' and $X(2)$ are large when $p_1 = q_1 = 0.05$ (Figures 2 and 3). Furthermore, F' has the property that when both p_1 and q_1 are low it becomes very large, *e.g.*, if $p_1 = q_1 = 0.01$, $F' = 24.5$, compared to when $p_1 = q_1 = 0.5$, then $F' = 0.25$.

Last, notice that in Figures 2 and 3 the values of F' and $X(2)$ may actually be negative. HEDRICK and

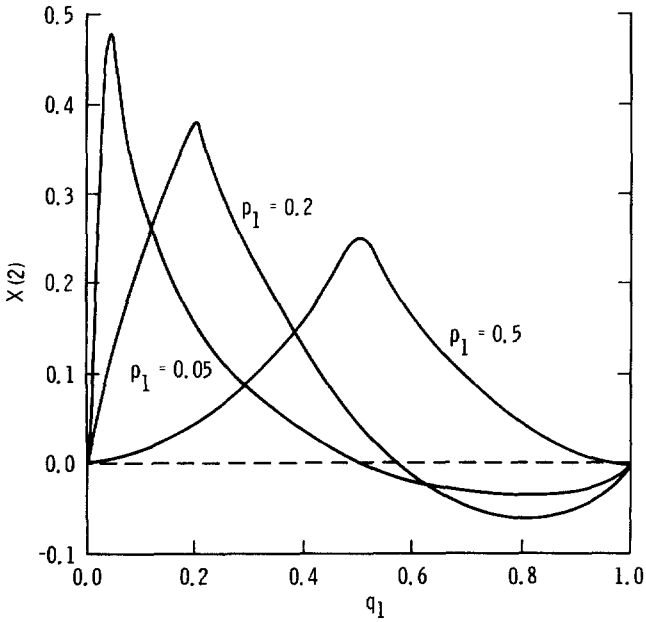

FIGURE 3.—Magnitude of $X(2)$ for different p_1 and q_1 values when $D' = 0.5$.

THOMSON (1986) showed that this occurs when there are two alleles at each locus for F^* when

$$-2D(p_1q_1 - p_1q_2 - p_2q_1 + p_2q_2) < 4D^2. \quad (16)$$

The same conditions are also true for F' and $X(2)$.

To determine the maximum value of these different measures for given allelic frequencies, expression (2) with the substitution $D'_{11} = |1|$, gives $D_{11} = D_{\max}$. Substituting the value of $D_{\max}$ in the various equations gives the maximum possible so, for example, the maximum D^* when $D_{11} > 0$ and $p_1(1 - q_1) < (1 - p_1)q_1$ becomes

$$D^* = \frac{p_1^2(1 - q_1)^2}{H_A H_B}. \quad (17)$$

To calculate the maximum F', expression (22) can be used.

When there are multiple alleles, the disequilibrium is intuitively highest when there are the same number of alleles at both loci ($k = l$) and $x_{ii} = p_i = q_i$ (all other gametes, $x_{ij} = 0$ where $i \neq j$), a situation termed absolute association by CLEGG *et al.* (1976). First, assuming only coupling gametes are present in the population and all are at equal frequency, *i.e.*, $p_i = q_i = 1/k$, then we can calculate the disequilibrium generated for the different measures (see Table 1). In this situation, $D^* = Q^* = F' = 1/(k - 1)$ and the extent of disequilibrium declines for these measures as the number of alleles increases. $X(2)$ and D' are equal to unity for any number of alleles. The dependence of D^*, Q^* and F' on the number of alleles at a locus makes these measures less useful when comparing samples with different numbers of alleles. This problem could be rectified by multiplying them by $k - 1$ when $k = l$ (remember YAMAZAKI'S measure L does this for Q^*).

TABLE 1

Amount of disequilibrium for the different measures when $k = l$, and all $x_{ii} = 1/k$

	$k(=l)$			
	2	3	4	k
D^2	0.25	0.222	0.1875	$\frac{k-1}{k^2}$
D^*	1.0	0.5	0.333	$\frac{1}{k-1}$
Q^*	1.0	0.5	0.333	$\frac{1}{k-1}$
F'	1.0	0.5	0.333	$\frac{1}{k-1}$
$X(2)$	1.0	1.0	1.0	1
D'	1.0	1.0	1.0	1

Second, let us assume that there is still absolute association and only coupling gametes but relax the assumption that all gametes have a frequency of $1/k$. Table 2 gives the values for the different measures for examples with 2, 3, and 4 alleles and general expressions for k alleles. The allelic frequencies chosen for the examples are ones that fit the expectations for a neutrality population with a given number of alleles (EWENS 1979). In this case again, $X(2)$ and D' are always unity and $Q^* = 1/(k - 1)$. On the other hand, D^* and F' do not have values solely dependent on the number of alleles as they did when all coupling gametes had equal frequencies.

Given that there are more than two alleles at both loci, and the frequencies of the alleles at the two loci are different, *i.e.*, $p_i \neq q_i$, then in general there is no way in which D' can be unity. However, for three alleles it is possible to have $D' = 1$ given that $p_1 \geq p_2 + p_3$ and $q_1 \geq q_2 + q_3$. Table 3 (center columns) gives an example of two such gametic arrays with $p_1 = 0.5$, $p_2 = p_3 = 0.25$ and $q_1 = 0.85$, $q_2 = 0.1$, and $q_3 = 0.05$ and similar ones given equal allelic frequencies at the two loci ($p_1 = q_1 = 0.85$, $p_2 = q_2 = 0.1$, and $p_3 = q_3 = 0.05$ in the left column and $p_1 = q_1 = 0.5$ and $p_2 = p_3 = q_2 = q_3 = 0.25$ in the right column). First, notice that even though the left and right columns of Table 3 have equal allelic frequencies at the two loci, the values of the measures except D' are quite different. In fact, in the first column where allelic frequencies are consistent with neutrality, both F' and $X(2)$ are negative while in the right column, they are positive. When the allelic frequencies are different at the two loci, then F' and $X(2)$ are still negative for the first array of gametic frequencies (column 2) but positive for the second array of gametic frequencies (column 3). In other words, given that the disequilibrium is the maximum possible for these allelic frequencies,

TABLE 2

Amount of disequilibrium for the different measures when $k(=l)$ and there is absolute association with $x_{ii} = p_i = q_i$ and the allelic frequencies at a locus differ[a]

	$k(=l)$			
	2 (0.92, 0.08)	3 (0.85, 0.1, 0.05)	4 (0.79, 0.15, 0.05, 0.01)	k $(p_1, p_2, p_3 \cdots p_k)$
D^2	0.0217	0.0440	0.0776	$\sum_{i=1}^{k} p_i^2(1-p_i)^2 + \sum_{i\neq j}^{k}\sum^{k} p_i^2 p_j^2$
D^*	1.0	0.637	0.630	$\dfrac{D^2}{\left(1-\sum_{i=1}^{k} p_i^2\right)^2}$
Q^*	1.0	0.5	0.333	$\dfrac{1}{k-1}$
F'	0.173	0.361	0.540	$\dfrac{\sum_{i=1}^{k} p_i^2 - \left(\sum_{i=1}^{k} p_i^2\right)^2}{\left(1-\sum_{i=1}^{k} p_i^2\right)^2}$
$X(2)$	1.0	1.0	1.0	1
D'	1.0	1.0	1.0	1

[a] The frequencies of the coupling gametes are given in parentheses.

TABLE 3

Amount of disequilibrium for different measures for the gametic arrays given

	$x_{11}\ x_{12}\ x_{13}$ $x_{21}\ x_{22}\ x_{23}$ $x_{31}\ x_{32}\ x_{33}$			
	(a)	(b)	(c)	(d)
	0.7, 0.1, 0.05 0.1, 0.0, 0.0 0.05, 0.0, 0.0	0.35, 0.1, 0.05 0.25, 0.0, 0.0 0.25, 0.0, 0.0	0.5, 0.0, 0.0 0.25, 0.0, 0.0 0.1, 0.1, 0.05	0.0, 0.25, 0.25 0.25, 0.0, 0.0 0.25, 0.0, 0.0
D^2	0.0012	0.0131	0.0306	0.141
D^*	0.0174	0.0792	0.185	0.360
Q^*	0.0078	0.0441	0.132	0.250
F'	−0.359	−0.0943	0.358	0.280
$X(2)$	−0.130	−0.0728	0.277	0.467
D'	1.0	1.0	1.0	1.0

then F' and $X(2)$ may either be positive or negative depending upon the particular gametic array.

Rate of decay: Another important property of a disequilibrium measure is the rate of decay of disequilibrium as indicated by the measure in an infinite or very large population. It is assumed here for simplicity that some factor such as a population bottleneck or hybridization of two populations generates some disequilibrium and then the decay of disequilibrium is only a function of the amount of recombination c between the two loci. It is important to note that disequilibrium can be generated continuously by such factors as genetic drift (*e.g.*, HILL and ROBERTSON 1968; OHTA and KIMURA 1969) and selection (*e.g.*, LEWONTIN and KOJIMA 1960) and the rate of decay may also be affected by inbreeding, the mode of reproduction, or selection (*e.g.*, HEDRICK 1980; P. W. HEDRICK, unpublished data, 1987; ASMUSSEN and CLEGG 1982).

A well known result (*e.g.*, HEDRICK 1983) is that

$$D_{ij\cdot t+1} = (1 - c)D_{ij\cdot t}. \tag{18}$$

In other words, the rate of decay per generation is the ratio of the disequilibrium in generation $t + 1$ to that in generation t so that for the measure D_{ij}, the rate of decay is $1 - c$. In addition, because

$$D_{ij\cdot t} = (1 - c)^t D_{ij\cdot 0} \tag{19}$$

the rate of decay over t generations for the measure D_{ij} is $(1 - c)^t$.

Because H_A, H_B, p_i, and q_j do not change as a result of recombination, then

$$D^2_{t+1} = (1 - c)^2 D^2_t \tag{20a}$$

$$D^*_{t+1} = (1 - c)^2 D^*_t \tag{20b}$$

$$Q^*_{t+1} = (1 - c)^2 Q^*_t \tag{20c}$$

and

$$D'_{t+1} = (1 - c)D'_t \tag{20d}$$

making the rate of decay per generation for D^2, D^* and Q^* equal to $(1 - c)^2$ and that for D' equal to $1 - c$. In addition

$$D^2_t = (1 - c)^{2t} D^2_0 \tag{21a}$$

$$D^*_t = (1 - c)^{2t} D^*_0 \tag{21b}$$

$$Q^*_t = (1 - c)^{2t} Q^*_0 \tag{21c}$$

and

$$D'_t = (1 - c)^t D'_0 \tag{21d}$$

TABLE 4

Rate of decay per generation for F' and $X(2)$ when there is absolute association of alleles (only coupling gametes)

Generation	$p_i = 1/k$ and/or $q_j = 1/l$	$k(=l)$ 2[a] $c = 0.05$	2[a] $c = 0.5$	3[a] $c = 0.05$	3[a] $c = 0.5$	4[a] $c = 0.05$	4[a] $c = 0.5$
1	$(1-c)^2$	0.942	0.457	0.939	0.443	0.934	0.415
2	·	0.942	0.476	0.940	0.467	0.934	0.449
3	·	0.942	0.488	0.940	0.483	0.935	0.471
4	·	0.943	0.494	0.940	0.491	0.935	0.485
5	·	0.943	0.497	0.941	0.495	0.936	0.492
10	·	0.944	0.499	0.942	0.500	0.938	0.500
20	·	0.946	·	0.945	·	0.942	·
40	·	0.949	·	0.948	·	0.947	·
80	·	0.950	·	0.950	·	0.950	·
Asymptotic	$(1-c)^2$	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$

[a] The initial gametic arrays used here were the same as in Table 2.

TABLE 5

Rate of decay per generation for F' and $X(2)$ for the four initial gametic arrays given in Table 3 when $c = 0.05$ or 0.5

Generation	(a) 0.05	(a) 0.5	(b) 0.05	(b) 0.5	(c) 0.05	(c) 0.5	(d) 0.05	(d) 0.5
1	0.952	0.512	0.990	0.710	0.926	0.371	0.889	0.179
2	0.952	0.506	0.986	0.574	0.926	0.413	0.888	0.050
3	0.952	0.503	0.983	0.532	0.927	0.447	0.887	−1.750
4	0.952	0.501	0.980	0.515	0.927	0.471	0.886	0.82
5	0.952	0.501	0.978	0.507	0.928	0.484	0.885	0.598
10	0.951	0.500	0.969	0.501	0.931	0.499	0.878	0.504
20	0.951	·	0.960	·	0.936	·	0.842	0.500
40	0.950	·	0.953	·	0.944	·	1.024	·
80	·	·	0.950	·	0.950	·	0.954	·
Asymptotic	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$	$(1-c)$

making the extent of decay for D^2, D^*, and Q^* over t generations equal to $(1-c)^{2t}$ and that for D' equal to $(1-c)^t$.

The rates of decay for F' and $X(2)$ are more complicated than the other measures but equal to each other. The basis for their equivalent rate of decay can be seen when $x_{ij} = D_{ij} + p_i q_j$ is substituted [from expression (1)] into expression (10) which then becomes

$$F^* = D^2 + 2\sum_{i=1}^{k}\sum_{j=1}^{l} p_i q_j D_{ij} + \sum_{i=1}^{k}\sum_{j=1}^{l} p_i^2 q_j^2 - \sum_{i=1}^{k} p_i^2 \sum_{j=1}^{l} q_j^2. \quad (22)$$

Notice that expressions (12) and (22) both have disequilibrium measures of the same sort and ratio, D^2 to $2\sum\sum p_i q_j D_{ij}$, indicating the reason for the same rate of decay for these measures. Of course, terms composed only of p_i and q_j combinations do not change as the result of recombination.

Because both F' and $X(2)$ are functions of both D^2 and D_{ij}, their rate of decay is a function of the initial gametic array. To illustrate their behavior, some examples are given in Tables 4 and 5. First, note in the first column of Table 4 that when all $p_i = 1/k$ and/or all $q_j = 1/l$ that the rate of decay per generation for F' and $X(2)$ is $(1-c)^2$ just as it is for D^2, D^* and Q^*. Second, in the remainder of Table 4 assuming still absolute coupling but unequal allelic frequencies, then the rate of decay changes but eventually asymptotes at $1-c$. In other words, with initial absolute disequilibrium the per generation rate of decay may be $(1-c)^2$ or $(1-c)$ depending upon the initial gametic array.

Finally, in Table 5 the rate of decay is given for the arrays in Table 3. Here, all the arrays eventually have a decay rate of $1-c$ but some start out in the early generations with a much slower decay rate, *e.g.*, array (*b*), while others have a much faster decay rate, *e.g.*, array (*c*). The array (*d*) has a most unusual rate of decay because both F' and $X(2)$ are initially negative but with decay, first become positive until generations 39 and 3 when $c = 0.05$ and 0.5, respectively, and then approach zero from the positive side. For example, the rate of decay for this array when $c = 0.5$, is first very large, then becomes a large negative value

TABLE 6

Statistics describing the distribution of six measures of total disequilibrium for $n = 200$

	$4Nc = 0$				$4Nc = 10$			
	$\bar{x}$	s	g_1	g_2	$\bar{x}$	s	g_1	g_2
$k = l = 2$								
D^2	0.014	0.044	3.86	14.3	0.002	0.008	7.09	66.9
D^*	0.130	0.295	2.38	4.02	0.029	0.085	6.23	51.1
Q^*	0.130	0.295	2.38	4.02	0.029	0.085	6.23	51.1
F'	0.661	5.36	12.6	200.	0.091	3.36	23.1	637.
$X(2)$	0.106	0.310	2.27	3.66	0.011	0.112	4.46	25.6
D'	1.000	0.013			0.913	0.225	−2.64	5.81
$4Nu = 0.25$								
D^2	0.027	0.054	2.26	4.18	0.006	0.013	3.98	19.8
D^*	0.166	0.269	1.79	2.07	0.043	0.081	4.54	31.8
Q^*	0.105	0.187	2.81	9.04	0.031	0.065	6.22	62.3
F'	0.486	2.95	18.3	500.	0.084	1.832	33.8	1620.
$X(2)$	0.173	0.330	1.37	0.43	0.032	0.127	2.65	9.87
D'	0.934	0.174	−3.08	9.18	0.772	0.301	−0.94	−0.60
$4Nu = 1$								
D^2	0.060	0.054	0.62	−0.73	0.018	0.019	1.49	2.56
D^*	0.194	0.161	1.16	1.63	0.062	0.054	2.27	10.1
Q^*	0.060	0.050	3.35	34.5	0.027	0.023	3.09	28.2
F'	0.319	0.609	14.7	549.	0.087	0.384	30.2	1690.
$X(2)$	0.344	0.293	0.09	−1.08	0.097	0.132	0.76	0.81
D'	0.833	0.184	−1.41	1.70	0.575	0.208	0.32	−0.27

for one generation, then a large positive value, and finally asymptotes to 0.5.

SIMULATION TECHNIQUES

In order to compare the overall values of these measures in an objective manner rather than for selected examples, random samples were obtained using the program of HUDSON (1983). These samples are of a specified size n from a population under neutrality equilibrium where N is the finite population size, u is the mutation rate to new alleles, and c is the recombination rate between the two loci. The samples were examined both conditioned on the number of alleles at the A locus (k) and the B locus (l) and unconditionally, *i.e.*, for all samples obtained from a given parameter set of $4Nu$ and $4Nc$. The conditional samples used were all larger than 1,000 while the unconditional samples were between 10,000 and 20,000. The results conditioned on allele number will focus on $k = l = 2$, the form in which most restriction site or base polymorphism data is generally observed. From extensive simulations, HUDSON (1985) has shown that for a sample from a neutrality population the disequilibrium values, conditioned on the number of alleles, are nearly independent of $4Nu$.

Distributions of measures: HEDRICK and THOMSON (1986) discussed at length the distribution of the disequilibrium measures, D^*, Q^* and F^*, conditioned on the number of alleles in a sample of size n, giving the mean, 95% intervals as well as the distributions of F^* and Q^* for a particular combination of parameters. One general conclusion from this examination was that these distributions generally have very large variances so that the 95% interval in the cases of D^* and Q^* extended from the minimum of zero to a quite large value. In addition, these measures all appeared to be right-skewed, *i.e.*, having long tails of high disequilibrium values. The 95% intervals were reduced as the number of alleles in the sample increased and as $4Nc$ became larger. The unconditional distribution of D^* was examined by MARUYAMA (1982) and he found that it had a large variance with a long right-hand tail. If $4Nc$ is small, say <0.1, then the distribution is slightly U-shaped because some samples are at maximum disequilibrium [see Figure 5 in MARUYAMA (1982)]. Both GOLDING (1984) and HUDSON (1985) investigated the conditional distribution of disequilibrium when there are only two alleles at each locus.

With this background, let us examine some of the distributional properties of the six different disequilibrium measures given above. As an overall perspective, Table 6 gives the distributional properties for samples of size 200 conditioned on $k = l = 2$ and unconditioned for $4Nu = 0.25$ and 1 when $4Nc = 0$ and 10. The statistics used are the mean ($\bar{x}$), standard deviation (s) and g_1 and g_2, measures of skewness and kurtosis, respectively, that have expectations of zero when the distribution is normal (*e.g.*, SOKAL and ROHLF 1981).

First, let us compare D^2, D^*, Q^*, and $X(2)$, a group of measures that we will find below to be generally highly correlated with each other. In fact, Q^* is correlated only when k and l are low, remember when $k = l = 2$, $D^* = Q^*$ (Table 6). For these four measures,

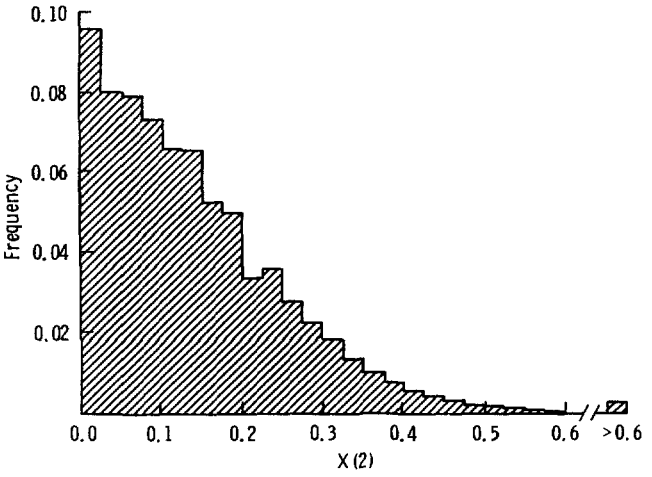

FIGURE 4.—Distribution of $X(2)$ when $n = 200$, $4Nc = 10$ and $4Nu = 1$.

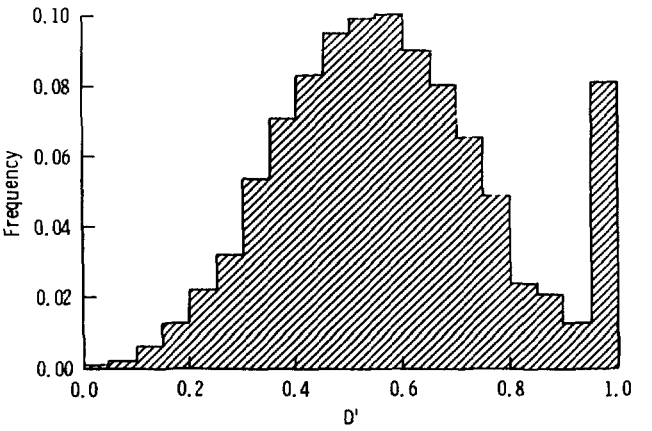

FIGURE 5.—Distribution of D' when $n = 200$, $4Nc = 10$ and $4Nu = 1$.

the standard deviation is the same general size as the mean or larger, indicating the extreme variance of these distributions. Note that when $k = l = 2$ the standard deviation of these four measures is much larger than the mean. Second, these measures show right skewness (positive g_1 values) with the largest g_1 values for Q^* and D^*. In other words, these measures have a few samples with much higher than the average disequilibrium, particularly for larger $4Nc$ values. Last, these measures generally have flatter distributions than normal distributions, *i.e.*, platykurtic with positive g_2 values, a characteristic that is more pronounced for higher $4Nc$ values. Overall, $X(2)$ is the measure of these four that is the least right-skewed and platykurtic, *i.e.*, the measure of these four having the most normal distribution.

The distributions of D^* and Q^* conditioned on sample size are similar to the distributions conditioned on both sample size and allele number and given in HEDRICK and THOMSON (1986). As an example of the distribution for $X(2)$, its distribution is given for $4Nu = 1$, $4Nc = 10$, and $n = 200$ in Figure 4. The 95% interval extends from 0.002 to 0.392, with a few values larger than 0.6.

The other two measures, F' and D', are different because F' can have very extreme values when H_AH_B is low and D' is a function of the normalized measure D'_{ij}. For example, notice that the mean value of F' is larger for $4Nu = 0.25$ than for $4Nu = 1.0$. The extreme right skewness and platykurtosis of F' is the result of these occasionally large F' values. D' has a smaller standard deviation relative to its mean than does the other five measures. The mean of D' is relatively near the maximum of unity for all cases in Table 6 except $4Nc = 10$ and $4Nu = 1$, and generally it shows a left skewness. In other words, when $4Nu = 0.25$ and $4Nc = 0$, most of the D' values are quite high but an occasional sample has low disequilibrium as measured by D', giving the left skewness. Figure 5 gives the distribution of D' when $n = 200$, $4Nc = 1$ and $4Nu = 1$. Notice the symmetry of the distribution of D' if the $D' = 1.0$ class is ignored, suggesting that along with $X(2)$ that D' is the most normally distributed of the measures.

Correlation of measures. The pairwise correlation coefficients of the six different total disequilibrium measures was calculated for samples from a population at equilibrium under neutrality for a wide range of values of n, $4Nu$ and $4Nc$. First, let us examine the correlation coefficients for a range of recombination values (keeping $4Nu$ and n constant) because we know that the disequilibrium values decrease as $4Nc$ increases (Table 6). One way to evaluate the pattern of these correlations is to focus on values above a given magnitude. As a guide, the correlations above 0.8 and between 0.6 and 0.8 are indicated in Table 7 (and subsequent tables).

First, note that in general the magnitude of the correlation between any given pair of measures, particularly the pairs with high correlations, declines as $4Nc$ increases. The major difference occurs between $4Nc = 10$ and $4Nc = 100$, suggesting that for the range between $4Nc = 0$ and $4Nc = 10$ (and maybe somewhat larger), the correlation patterns are consistent. The highest correlations are between the measures D^2, D^* and $X(2)$. If we look at the values of $4Nc = 0$, 1, and 10, all of the correlations between these measures are above 0.6 and 7 of 9 are above 0.8. In other words, these three measures form a group that appear to give much the same information concerning disequilibrium.

In addition, Q^* has a fairly high correlation with these three measures. The main difference between Q^* and these other measures is that Q^* is divided by the degrees of freedom. This appears to be the main reason why it does not have as high a correlation as the other members of this cluster (see discussion below). The other two measures, F' and D', have very

TABLE 7

Correlation coefficient between six total disequilibrium measures for four levels of recombination when $4Nu = 1.0$ and $n = 200$

		$4Nc$			
		0	1	10	100
D^2	D^*	0.83**	0.85**	0.82**	0.67*
	Q^*	0.44	0.47	0.40	0.20
	F'	0.17	0.21	0.14	0.09
	$X(2)$	0.86**	0.85**	0.74*	0.41
	D'	0.51	0.45	0.20	−0.05
D^*	Q^*	0.59	0.60*	0.53	0.40
	F'	0.46	0.51	0.40	0.50
	$X(2)$	0.87**	0.87**	0.79*	0.54
	D'	0.48	0.45	0.29	0.22
Q^*	F'	0.48	0.51	0.45	0.43
	$X(2)$	0.60*	0.62*	0.59	0.44
	D'	0.24	0.21	0.06	0.00
F'	$X(2)$	0.45	0.51	0.46	0.63*
	D'	0.08	0.08	−0.05	−0.19
$X(2)$	D'	0.45	0.38	0.06	−0.22

* <0.8 and >0.6; ** >0.8.

TABLE 8

Correlation between the six total disequilibrium measures for three sample sizes when $4Nu = 1$ and $4Nc = 10$

		n		
		20	200	2000
D^2	D^*	0.75*	0.82**	0.84**
	Q^*	0.46	0.40	0.44
	F'	0.21	0.14	0.20
	$X(2)$	0.78*	0.74*	0.75*
	D'	0.12	0.20	0.25
D^*	Q^*	0.78*	0.53	0.46
	F'	0.67*	0.40	0.41
	$X(2)$	0.81**	0.79*	0.79*
	D'	0.30	0.29	0.33
Q^*	F'	0.76*	0.45	0.38
	$X(2)$	0.74*	0.59	0.57
	D'	0.13	0.06	0.06
F'	$X(2)$	0.65*	0.46	0.55
	D'	0.03	−0.05	−0.09
$X(2)$	D'	0.04	0.06	0.07

* <0.8 and >0.6; ** >0.8.

low correlations with the other measures and with each other (no values above 0.6).

Second, let us examine the effect of different sample sizes on the correlation coefficient for given $4Nu$ and $4Nc$ values. Table 8 gives these correlations for a 100-fold range in sample size from $n = 20$ to $n = 2000$. Notice again D^2, D^*, and $X(2)$ form a cluster of high values with Q^* being slightly lower. Importantly, the correlations between the measures in the high cluster appear to be independent of sample size and remain high over the 100-fold range in sample size.

TABLE 9

Correlation coefficient between the six total disequilibrium measures for three levels of mutation when $4Nc = 10$ and $n = 200$

		$4Nu$		
		0.25	0.5	1.0
D^2	D^*	0.69*	0.77*	0.82**
	Q^*	0.44	0.44	0.40
	F'	0.06	0.09	0.14
	$X(2)$	0.58	0.66*	0.74*
	D'	−0.14	−0.07	0.20
D^*	Q^*	0.84**	0.70*	0.53
	F'	0.45	0.43	0.40
	$X(2)$	0.87**	0.84**	0.79*
	D'	−0.03	0.04	0.29
Q^*	F'	0.53	0.50	0.45
	$X(2)$	0.77*	0.68*	0.59
	D'	−0.06	−0.04	0.06
F'	$X(2)$	0.44	0.48	0.46
	D'	−0.04	−0.07	−0.05
$X(2)$	D'	−0.19	−0.16	0.06

* <0.8 and >0.6; ** >0.8.

TABLE 10

Correlation between the six total disequilibrium measures when $k = l = 2$ for four combinations of n and $4Nc$

		n			
		20		200	
		$4Nc$			
		0	10	0	10
D^2	D^*, Q^*	0.76*	0.71*	0.76*	0.61*
	F'	0.13	0.22	0.04	0.03
	$X(2)$	0.74*	0.63*	0.74*	0.50
	D'	0.01	0.01	−0.32	−0.17
D^*, Q^*	F'	0.70*	0.83**	0.43	0.60*
	$X(2)$	0.99**	0.95**	0.99**	0.91**
	D'	0.01	0.11	0.01	−0.07
F'	$X(2)$	0.71*	0.84**	0.44	0.55
	D'	0.01	0.00	0.01	−0.02
$X(2)$	D'	0.01	−0.04	0.01	−0.16

Third, let us examine whether the correlations between the measures are dependent upon the mutation rate. Table 9 gives the correlations of the disequilibrium measures over a four-fold range of $4Nu$ for $n = 200$ and $4Nc = 10$. Again the highest correlations are between D^2, D^*, and $X(2)$ with Q^* having relative higher correlations with D^* and $X(2)$ when 4 Nu is lower. This latter result is of course due to fewer alleles being present when $4Nu$ is low so that the degrees of freedom does not influence Q^* very much.

Finally, let us examine the correlation between the measures when $k = l = 2$. Table 10 gives these values for four combinations of n and $4Nc$ (remember for k

TABLE 11

Summary of properties of different gametic disequilibrium measures for $k = l = 2$

	Range			Decay Rate	
	Minimum	Maximum	Comments	Per generation	Comments
D^2	0	$f(p_i,q_j)$		$(1-c)^2$	
D^*	0	$f(p_i,q_j)$		$(1-c)^2$	
Q^*	0	$f(p_i,q_j)$		$(1-c)^2$	
F'	$f(p_i,q_j)$	$f(p_i,q_j)$	May be negative; may be very large	Variable	Equal to $X(2)$
$X(2)$	$f(p_i,q_j)$	$f(p_i,q_j)$	May be negative	Variable	Equal to F'
D'	0	1	Independent of p_i,q_j	$(1-c)$	

$= l = 2$, $D^* = Q^*$, making the correlation between these two measures equal to unity). Notice here that the correlation between D^* or Q^* and $X(2)$ is very high, greater than 0.9 in all cases. The correlation with D^2 is somewhat lower, that with F' lower yet, and that with D' is near zero in all cases.

CONCLUSIONS AND DISCUSSION

Some properties of the six gametic disequilibrium measures discussed are summarized in Table 11 when there are two alleles at each locus. First, only D' is frequency independent and has the same range for all allelic frequencies. Particularly, when comparing samples with different allelic frequencies or different pairs of loci (see below) a frequency-independent measure is quite important. The maximum for all the other measures is a function of the allelic frequencies and the maximum for F' can be very large in some instances. In addition, the minimum of both F' and $X(2)$ has the unfortunate property of being negative.

An example of a situation in which these characteristics may be quite important is when using gametic frequencies to infer "recombinational hot spots" (*e.g.*, CHAKRAVARTI *et al.* 1984). Let us assume that four loci or restriction sites, A, B, C and D, are tightly linked and that there is the maximum disequilibria present possible for the observed allelic frequencies. Two such gametic arrays are given in Table 12 with the calculated disequilibrium values. These arrays were chosen so that the frequencies of the alleles at loci A and B were equal and those at loci C and D were equal but different from A and B. For example, for the array given on the left, the frequency of A_1 and B_1 are 0.2 and that of C_1 and D_1 are 0.4. Notice that the disequilibrium values for all the measures except D' are smaller between loci B and C than for locus pairs A–B and C–D. If one did not know that all the measures but D' were dependent upon allelic frequencies, then it would appear that the disequilibria between B and C was actually lower than for A–B and C–D. Such values have been used to suggest the presence of a recombinational hot spot which in this case may be only an artifact of the disequilibrium measure.

TABLE 12

Value of pairwise disequilibrium for the different measures for the frequency of four-locus gametes given in parentheses

	$(A_1B_1C_1D_1, A_1B_1C_2D_2, A_2B_2C_1D_1, A_2B_2C_2D_2)$					
Frequency of gametic array:	(0.2, 0.0, 0.2, 0.6)			(0.2, 0.0, 0.6, 0.2)		
Locus pair:	*A-B*	*B-C*	*C-D*	*A-B*	*B-C*	*C-D*
D^2	0.102	0.006	0.102	0.230	0.058	0.102
D^*	1.0	0.062	1.0	1.0	0.375	1.0
Q^*	1.0	0.062	1.0	1.0	0.375	1.0
F'	2.125	−0.219	2.125	1.083	0.562	2.125
$X(2)$	1.0	−0.103	1.0	1.0	0.370	1.0
D'	1.0	1.0	1.0	1.0	1.0	1.0

Another situation in which a frequency-dependent measure may lead to erroneous conclusions is in the examination of the effect of an evolutionary factor on disequilibrium. For example, FUERST and MARUYAMA (1986) used D^* to examine the effect of population bottlenecks on gametic disequilibrium and came to the conclusion that the extent of disequilibrium depended upon the allelic frequencies after the bottleneck. However, the measure D^* is itself a function of the allelic frequencies so it is probable that their conclusions are not due to the population bottleneck but to an artifact in the disequilibrium measure they used.

Second, as stated in Table 11 the rate of decay due to recombination per generation in a large population is constant for D^2, D^*, Q^* and D' being the smallest. The rate of decay in this situation for F' and $X(2)$ varies over time and asymptotes at $(1-c)$ or $(1-c)^2$ depending upon the initial gametic array.

Some general properties of the six measures are summarized in Table 13. For example, when samples from a population at equilibrium under neutrality are examined, in general F' has the largest variance (and g_1 and g_2 values) and D' the lowest variance. Overall D^2, D^*, $X(2)$ and to some extent Q^* form a cluster of fairly highly correlated measures. F' is not part of the cluster, apparently because of the very large values that this measure may have. D' is uncorrelated with the other measures apparently because it is allelic-

TABLE 13

Summary of general properties of the different gametic disequilibrium measures

	Distribution	Correlation	Biological	Statistical
D^2		High with D^*,$X(2)$		
D^*		High with D^2,$X(2)$		
Q^*				
F'	High s, g_1,g_2		Related to homozygosity	
$X(2)$		High with D^2,D^*	Related to variance of heterozygosity	Test available
D'	Low s	Uncorrelated		

frequency independent while all of the others have frequency dependence. Both F' and $X(2)$ have some biological relevance (Table 13) while the other measures have no particular relationship to biological entities such as homozygosity or heterozygosity. Additionally, $X(2)$ has a statistical advantage because of the presence of a statistical test (BROWN, FELDMAN and NEVO 1980).

From these considerations, in my opinion, one should proceed with caution when using a particular gametic disequilibrium measure. One should be careful to use a truly allelic frequency-independent measure such as D'. The normalized measures D^*, Q^*, F' and $X(2)$ are very frequency dependent. For example, the good biological and statistical properties of $X(2)$ may be outweighed by its frequency-dependence, negative values, and variable rate of decay. Furthermore, the traditional propensity towards D^2 and D^* because of mathematical tractability may be less important than their strong frequency dependence. As a result, prudence would suggest that D' or some such frequency-independent measure of disequilibrium should be used to insure confidence in conclusions based on statistical associations of alleles at two (or more) loci.

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